Report of the Haffkine Institute

For the Year 1931

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REPORT OF THE HAFFKINE INSTITUTE FOR THE YEAR 1931

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Mr. S. N. Gore, L.M. & S. (Bom.).

Supernumerary Officer.

Major S. D. S. Greval, B.Sc. (Punj.), M.D., Ch.B., D.P.H., I.M.S., acted as Assistant Director from 6th June to 2nd July 1931, and Supernumerary Officer from 3rd July to 31st December 1931.

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Mr. R. G. Sathe, G.B.V.C., Veterinary Assistant Surgeon.

Mr. E. R. Nagarajan, G.M.V.C., Veterinary Assistant Surgeon.

Inquiry on Maternal Morbidity and Mortality in Childbirth.

Dr. Margaret Balfour, C.B.E., M.D.
Dr. Lucy Wills, M.D., from 1st January to 24th February 1931.
Mrs. S. K. Talpade, M.B., B.S.

Inquiry on Sprue and Morbid Histology of the Intestinal Tract.

Dr. F. Walwyn, M.D., from 1st April to 30th September 1931.

Tuberculosis Inquiry.

Dr. M. B. Soparkar, B.Hy., M.D. (Bom.).
Jamadar C. S. Dhilon, I.M.D.

Inquiry on the Serological Reactions of B. avisepticus.

Dr. P. M. Wagle, M.B. (Bom.), D.Bac. (Manch.), D.P.H. (Lond.), D.T.M. (L'pool).

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Mr. A. Petty—(Plague Vaccine).
Mr. J. Mallett—(Plague Vaccine).
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Junior staff, subordinates and menials number 118.

General Review.

The Institute continued to function during the year as (*a*) the centre for manufacture of plague vaccine for the whole of India, (*b*) the Provincial Bacteriological Laboratory for the Bombay Presidency, (*c*) the centre for manufacture of antirabic vaccine for Bombay Presidency and adjoining areas, and treatment centre for Bombay City and, in addition, carried out research work on plague and other subjects as in former years, largely with the assistance of the Indian Research Fund Association.

Plague Work.

(1) *Antiplague vaccine.*—The manufacture of the vaccine has, as in former years, constituted the main activity of the Institute. The demands for vaccine continue to be high in proportion to the incidence of plague mortality, and the slight rise in plague during the year over the number of cases occurring during 1930 was reflected in an increase in issues. It has been shown in previous reports that a regular increase in demand in relation to cases occurring has continued in successive years, and the proportion of issues in the present year has been 31 doses of vaccine for each plague death registered. The proportionate demands are shown in the chart. A fresh interest in the value of antiplague inoculation has recently been shown and a special enquiry into the subject has been made by the *Comite de l'Office International d'Hygiene Publique.* Reports on the results obtained with vaccines in different countries have been submitted to the Committee including one from this Institute in which the plague inoculation statistics for India have been fully set out. These reports have been the subject of discussion at the meetings of the Committee in Paris and it has been made evident that the vaccine prepared at Parel has given better results than that prepared elsewhere. As a result of this investigation there have been enquiries from other countries as to the possibility of supply of vaccine from the Haffkine Institute, and in regard to the methods of preparation of the vaccine adopted and the strains of *B. pestis* used. During recent years the institution of a fresh experimental investigation into the vaccine and its properties, and into the effect of each procedure employed in preparation has resulted in undoubted improvements. Experimental evidence has shown an increase in its immunising value as the result of the use of revised methods. Such statistics as are available as to the protective value of vaccine prepared in recent years as compared with older results also suggests a marked increase in potency. Proposals have been put up for a statistical enquiry on exact lines for determining the value of inoculation with the vaccine in its present form.

(2) *Researches in connection with plague vaccine.*—The methods introduced by Dr. Gore for the preparation of pure strains of *B. pestis* from passage animals for seed purposes have been found to be fully effective. The differential methods for determination of purity which are now used have ensured that the losses of vaccine under preparation which formerly occurred due to the use of impure seed could be completely avoided.

Dr. Gore has continued his work on the study of contaminants and has in addition shown the value of differential growth methods for the purpose of distinguishing *B. pseudo-tuberculosis rodentium* from *B. pestis*. The value of a copper sulphate culture medium for the growth of *B. pestis* has been studied and has given promising results.

Major Sokhey and Dr. Maurice have continued their study of the influence of various factors on the value of the vaccine. The relationship of virulence to potency and toxicity to potency have been studied and also the effect of the period of incubation, temperature of incubation, reaction and composition of medium on the immunising value of the vaccine. The question of a technique for biological standardisation of the vaccine has been the subject of further study. A prolonged series of experiments with different brews of vaccine has given consistent results, but at intervals difficulties have been experienced due to changes in virulence of the passage strain of *B. pestis*. It is hoped that when this difficulty can be overcome the method of testing will afford a reliable means of ascertaining the value of successive brews and of comparing the potency of vaccines prepared by variations in technique by entirely different methods. The technique has been applied to the comparison of the relative value of Haffkine's vaccine to agar-grown and "derived" vaccines prepared by other laboratories. The results of some of these tests are given in the research section of this report and only one vaccine has been found to compare with the Haffkine vaccine, this being a vaccine of high bacterial content and toxicity. Chemical studies of the changes occurring in the vaccine during the period of incubation have been carried out with the object of ascertaining the probable immunising fraction.

(3) *Epidemiology of Plague.*—A systematic investigation into the immunity of rats to plague in areas in which the disease has occurred in epidemic and epizootic form has been commenced during the year. This immunity is roughly proportional to the incidence of the disease and it is intended to examine rats from the different provinces of India to observe the progress of development of immunity as tested against the standard dosage of *B. pestis*. A repetition of these tests at intervals may furnish a valuable clue to the future progress of plague. The first tests done during the year show a high degree of immunity in rats from various places in the Bombay Presidency.

(4) *Antiplague Serum.*—Dr. Naidu and his assistants have continued their work on the preparation of antiplague serum using a variety of animals with the object of determining the type most suitable for the work. Many of the sera prepared have been of high potency as tested on experimental animals and a trial on a short series of human cases has given very promising results. Work has been commenced on concentration of the serum and it is hoped in the near future to have an opportunity to have an extended trial.

(5) *Chemotherapy of Plague.*—The work with the halogenomercuriphenols has been continued during the year with inconclusive results and was considerably handicapped by a shortage of suitable animals.

Certain indications of reduction of septicaemia and prolongation of life following the administration of some of the compounds of the series were noted but no definite indication of life saving value.

The Pharmacological Unit.

The principal investigation carried out during the year by Father Caius and Dr. Mhaskar was in connection with scorpion stings. The properties of the scorpion venom were very fully studied. The toxicity of the venom of various species was determined in relation to body weight and the conclusion arrived at that a scorpion sting could in no case be fatal to a human being. The various traditional Indian plant remedies for scorpion bite were investigated and found to be without value. The investigation of the value of a number of indigenous drugs reputed to be of use in eye diseases and other conditions was also carried out and the results will form the subject of future publications.

The Biochemical Unit.

In addition to routine biochemical examinations the unit undertook the standardisation of all media used in the preparation of the vaccine and also carried out further investigations into the changes occurring in the broth during the process in incubation. The results of this work are given in a later section.

An extensive investigation was undertaken into the normal standards of chemical constituents of the blood of adult Indians in order to establish a basis for a future investigation into the changes occurring in the blood in kidney and other diseases in persons whose dietary and, in particular, whose protein intake differs widely from that used in Western countries for which standards have been established.

The Antirabic Department.

The number of cases of dog-bite receiving prophylactic treatment has, as in former years, shown a progressive increase. The total dealt with at the Institute rose to 1,061 with a mortality of 0·28 per cent. which compares very favourably with that of other Institutes in India. The results for the last two years since the introduction of the use of the Paris strain of fixed virus and graded courses of treatment according to the assessment of risk have been the best recorded. A corresponding improvement has not taken place in regard to cases treated at out-centres. The majority of the Institute cases are from Bombay City itself and the early stage at which cases attend for treatment and the thorough immediate disinfection measures adopted may be responsible in some degree for the better results obtained. The first case of a "paralytic accident" amongst those treated with vaccine prepared at this Institute was recorded during this year. The case fortunately ended in complete recovery.

Inquiry on Maternal Morbidity and Mortality in Childbirth.

This enquiry was unfortunately reduced to a very small scale owing to the departure of certain members of the staff and the difficulties of financing further work. Dr. Talpade continued the investigations into the dietaries of certain classes of pregnant women and followed up their after-histories. The results of this work were of considerable interest and are now under analysis.

Tuberculosis Enquiry.

Dr. Soparkar and his assistants continued their investigations on tuberculosis during the year and studied a large number of strains of *B. tuberculosis* from human and cattle cases. They have investigated the question of the filterability of the bacillus, the variations in its character and the relationship of human and bovine disease. The survey of the incidence of tuberculosis as determined by the Von Pirquet test was extended to the Madras Presidency and neighbouring States in order to obtain figures to compare with those for the Bombay Presidency which had been collected during the previous year. An extract of the findings is given in a later section.

Minor Enquiries.

Major Anderson and Dr. Wagle continued their serological studies on the variants of *B. avisepticus*. Dr. Billimoria and Dr. Walwyn carried out the histological examinations of intestinal tissues required to complete the studies on sprue which had been undertaken by Colonel F. P. Mackie. A short investigation into the incidence of Leptospiral infection in rats in Bombay was undertaken by Major Anderson and Dr. Wagle.

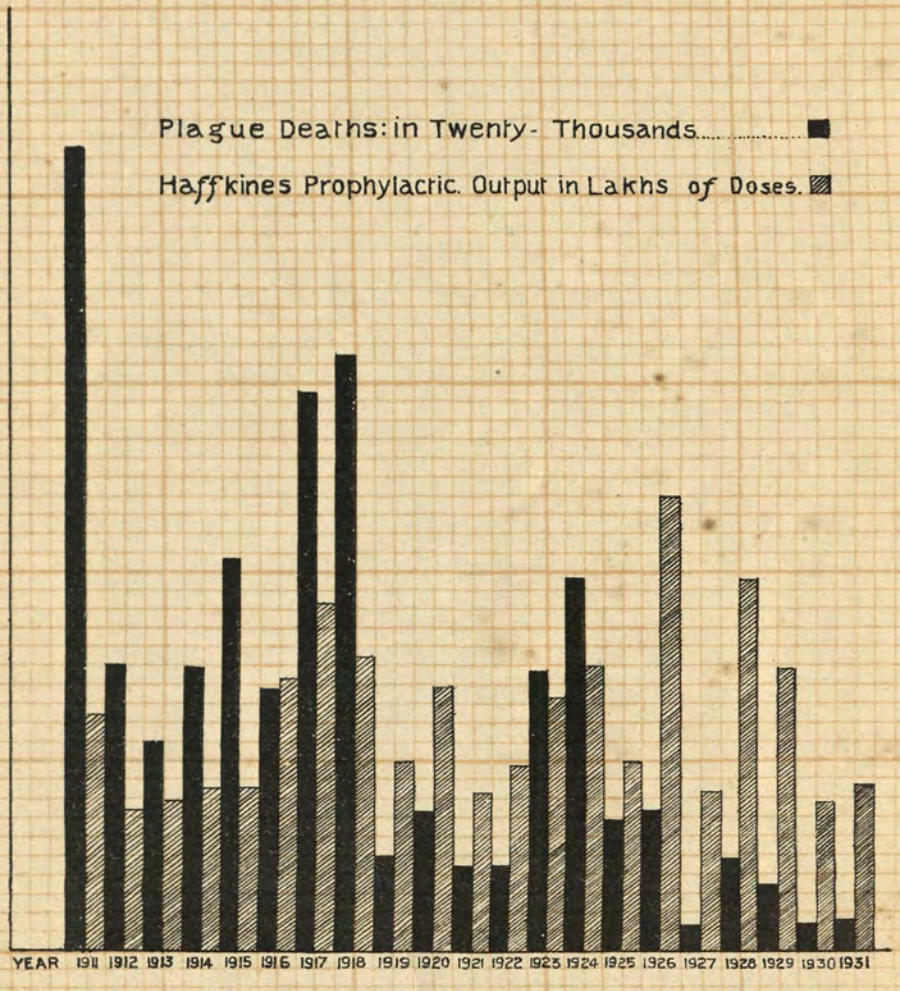
HAFFKINE'S PLAGUE PROPHYLACTIC VACCINE.

(1) *Manufacture.*—The value of antiplague inoculation is now universally recognized. The popularity of this measure is reflected in the output from this Laboratory, which has shown a steady increase in proportion to the incidence of plague.

From the opening of the Laboratory to the end of 1931, 34,273,979 doses have been sent out. The output of the vaccine has been as follows :—

Years.	Doses.
1897–1901	2,867,041
1902–1906	3,455,537
1907–1911	3,584,262
1912–1916	4,567,366
1917–1921	6,667,894
1922–1926	7,202,636
1927–1931	5,929,243
Total	34,273,979

Plague and the Out put of Haffkine's Plague Prophylactic.



G.P. Z.D. Poona, 1933.

During the year 1931 the output was 882,640 doses. The number of doses of antiplague vaccine despatched monthly during the past two years are detailed below :—

Month	*1930*	*1931*
January	94,074	19,196
February	125,982	63,594
March	51,330	128,718
April	25,870	73,290
May	6,408	6,018
June	1,464	17,838
July	19,176	17,724
August	68,436	71,172
September	67,992	90,064
October	107,616	90,672
November	100,752	65,874
December	104,410	138,480
Total	773,510	882,640

(2) *Distribution.*—The geographical distribution of the vaccine in India and to places outside India during the year 1930 and 1931 is shown below :—

—	Number of doses	
	1930	1931
British India.		
Bombay Presidency	123,206	107,333
Madras Presidency	68,940	84,296
Bengal, Bihar and Assam	5,368	51,552
United Provinces of Agra and Oudh	61,110	211,266
Punjab	16,164	31,834
North West Frontier Province	....	....
Central Provinces and Berar	16,236	42,204
Delhi Province	6	138
Burma	57,534	81,816
	348,564	610,439
Indian States.		
Southern Maratha Country	26,100	36,291
Sawantvadi	102	102
Baroda	102	....
Cutch	504	24
Hyderabad Division	135,276	66,438
Mysore	187,226	146,532
Benares	504	102
Patiala	....	600
Jammu	300	11,030
Bharatpur	....	1,200
Jaipur	1,002	....
Jodhpur	....	36
Rampur	504	6,612
Fategarh	....	96
Kathiawar	....	162
	351,620	269,225
Portuguese Territory in India	30	150

	Number of doses	
	1930	1931
Countries outside India.		
London	360	....
Capitob	....	24
China	2,802	2,652
Colombo	....	150
Baghdad	70,134	....
	73,326	2,976
Total number of doses ..	773,510	882,640

Examination of Rats for Plague.

The daily examination of rats collected by the Municipality has been continued during the year. Epizootic plague, as observed by examination of rats at the Institute, has never been absent from Bombay since the first introduction of the disease in 1896. The epizootic is now at a very low level, although few days pass without some plague infected rats being found. In the present year 748 were diagnosed plague, as compared with 8,476 in 1923 in which year the figures were compiled in their present form. The human plague deaths in 1923 and 1931 were respectively 928 and 20. The present low level of the epizootic and consequent low incidence of human cases is probably dependent on the resistance to plague infection which the Bombay rats now show. This is at present the subject of investigation.

Rats received at the Institute from the Municipality during 1931.

Month	Alive	Dead	Total
January	13,143	37,454	50,597
February	12,522	36,026	48,548
March	12,931	36,584	49,515
April	14,569	34,907	49,476
May	15,976	31,364	47,340
June	15,666	32,048	47,714
July	14,524	37,098	51,622
August	17,996	34,817	52,813
September	19,270	32,990	52,260
October	21,683	37,479	59,162
November	20,777	36,221	56,998
December	21,291	38,709	60,000
Total ..	200,348	425,697	626,045

Examination of dead rats for Plague during 1931.

Month.	Dead rats examined.	Plague infected rats.	*Rattus norvegicus.*	*Rattus rattus.*
January	27,581	43	42	1
February	26,386	40	39	1
March	23,750	77	68	9
April	22,120	136	125	11
May	20,672	109	105	4
June	21,322	41	40	1
July	26,468	51	51	..
August	23,624	54	54	..
September	21,746	44	43	1
October	27,364	45	38	7
November	22,051	46	38	8
December	27,232	62	56	6
Total ..	290,316	748	699	49

Relation of human plague deaths to plague deaths among rats.

Month.	Human plague deaths.	Rats examined post-mortem.	Plague infected rats.	Percentage mortality.
January	1	27,581	43	0·2
February	1	26,386	40	0·2
March	3	23,750	77	0·3
April	7	22,120	136	0·6
May	6	20,672	109	0·5
June	..	21,322	41	0·2
July	..	26,468	51	0·2
August	..	23,624	54	0·2
September	..	21,746	44	0·2
October	1	27,364	45	0·2
November	1	22,051	46	0·2
December	..	27,232	62	0·2
Total ..	20	290,316	748	0·26

Pharmacological Unit.

A. *Indigenous Drugs.*

1. Indian Plant Remedies used in scorpion-sting were examined in the course of the year. One hundred and ninety-five individual plants and 33 combinations were tested in complete detail. All the remedies, excepting 26, were also reputed snake-bite remedies. The finding with regard to these preparations is that none of the Indian Plants recommended for the treatment of scorpion-sting has any preventive, antidotal, or therapeutic effect.

2. The study of the composition and therapeutic value (*a*) of the bark of *Symplocos racemosa* Roxb., and of *Alstonia scholaris* R. Br., (*b*) of the dried rind of the fruit of *Garcinia Mangostana* Linn., (*c*) of the leaves of *Hydrocotyle asiatica* Linn., and *Melia Azadirachta* Linn., and (*d*) of the root of *Aconitum heterophyllum* is in progress, and will form the subject of a future publication.

The following were tried for their therapeutic effect in trachoma corneal ulcers and conjunctivitis :—Root-stock of *Curcuma longa* Linn., and *Berberis asiatica* Roxb., and roots of *Cissampelos Pareira* Linn.

3. Dr. K. S. Mhaskar read before the Pharmacological Section of the 22nd All-India Ayurvedic Conference held in Gwalior in December 1931, Papers on " Ophthalmology of the Ayurvedists ", and " Pharmacological Action of *Aconitum heterophyllum* Wall., on the circulatory system ". The Conference expressed its high appreciation of the Ayurvedic Research Work carried out at the Pharmacological Laboratory of the Haffkine Institute, Bombay, and at the Tropical School, Calcutta.

B. *Earth-eating and Salt-licking.*

Seven samples of soil from Salt-licks in India were analysed and some of the results have been sent for publication.

C. *Scorpion Venom.*

Owing to Government Resolution, Revenue Department, No. 3260/28, dated the 28th October 1929, regarding the transmission of scorpions by post, the extended study of scorpions and their venoms which it had been hoped to make, had to be reduced to a more limited series of observations. The notes form the subject of Memoir No. 24 of the Indian Medical Research Memoirs, and may be summarised as follows :—

The scorpions more commonly met with in India belong to either genus *Buthus* or genus *Palamnaeus*.

Scorpions never sting unless disturbed, and on this count all accidents are due to *Buthus* or, very exceptionally, to *Isometrus*.

The amount of venom a scorpion can inject in any animal larger than its prey never exceeds a few drops.

Scorpion-sting is very rarely fatal ; and thus scorpions are no more dangerous to human beings than bees or wasps.

It not unfrequently happens that the glands are partly, if not entirely, empty ; and, when full, there is a great difference between the quantities of venom contained in the individual glands of the same species.

Different animals exhibit different degrees of resistance : the cold-blooded frog is more resistant than mammals ; the dog, the rabbit, and the guinea-pig are more sensitive than the mouse or the bird ; the wild Indian rabbit is three times more resistant than the pure bred English rabbit ; the white rat and the white mouse are less resistant than the brown house rat.

Whatever the experimental animal injected, *Palamnaeus* venom is far less toxic than *Buthus* venom; and if dose for dose the time taken by the animal to die after the injection is reckoned as a measure of toxicity, this varies not only generically but also specifically.

The venom of the young scorpion is much less toxic than that of the adult.

The maximum amount of venom found in the glands of *Buthus tamulus* was 5·2 mg. If the toxicity of the venom in relation to body-weight was the same for man as for the very susceptible English rabbit, the total above quantity would not be lethal for a greater body-weight than 2·6 kilograms. Scorpion venom should then not be lethal to man, children not excepted.

As a result of poisoning, the nervous system becomes highly irritable; general excitability and the reflexes are increased; shivering, tremors, and muscular twitchings accompanied by disordered spasmodic movements are in evidence long before the appearance of paresis or paralysis of the individual muscles. The convulsions, when they occur, are very similar to those induced by strychnine. During the last stage of paralysis of the voluntary muscle, the motor nerve endings are affected much in the same way as by curare. Through its local irritant action the venom stimulates the terminations of the sensory nerves and produces excessive pain and a burning sensation.

After entering the circulation the venom acts on various centres in the bulb including the vaso-motor centre. The centres of the facial nerves are stimulated: there follows excessive lachrymal, nasal, and salivary secretion. The effect on the vagus centre and spinal centre results in secretion of laryngeal and tracheal mucus, and also in constriction of the larynx and the pharynx. The action on the centres of the cord is evidenced by the manifestation of painful general spasms.

The action on the nerve plexuses in the musculature of the intestine and the urinary bladder results in spasmodic contractions of those muscles; there is an increase in tone as well as in amplitude of rhythmical contractions. On the smooth muscle, the venom appears to act like the pilocarpine group by stimulating the nerve endings belonging to the parasympathetic system. The symptoms of vomiting and purging are thus accounted for.

The venom stimulates the heart which continues to beat after failure of the respiratory centre, and finally stops in midsystole or complete systole. The stimulation of the heart and the rise of blood-pressure, which are both independent of the central or vagus control, are most marked with *Buthus* venoms. The vaso-constriction is of peripheral origin and contributes in part to the rise of blood-pressure.

Death is entirely due to paralysis of the respiratory centre through the direct action of the venom, and is not dependent on the vagus centre, or the vagal nerve, or its endings.

Scorpion venoms are very complex liquids which consist of the following active principles: (1) neurotoxins acting principally (*a*) on the

respiratory centre and partly on (*b*) the vaso-motor centre, (*c*) on the nerve end plates in striated muscle, (*d*) on plexuses in the unstriated muscle, and (*e*) on the responsive mechanism of the parasympathetic and the sympathetic in the secretory glands; (2) haemolysins, agglutinins, haemorrhagins, leucocytolysins, coagulants, ferments, lecithin, and cholesterin; (3) a cardiac tonic; (4) a vascular tonic.

The agglutinins present in the venom point to important generic differences; and the haemolysins indicate specific variations.

Scorpion venom closely resembles snake venom.

The antivenom prepared at Kasauli against cobra and daboia venoms imparts a certain amount of protection to rabbits and dogs which have received lethal doses of the venoms of *Buthus tamulus* and *Palamnaeus swammerdami.*

Biochemical Unit.

1. *Chemical analyses of Haffkine's Prophylactic.*—Determinations outlined in the last report were continued during the year. In addition to analysing vaccine incubated at 27°C., vaccines incubated at 37·5°C. were also analysed.

Apart from the production of bacterial substance which can be separated from the liquid medium by filtration, the most constant change during the process of incubation is the addition to the medium of a heat coagulable protein, which fraction, when separated by precipitation with acetic acid, does have protective power. This fraction has not yet been separated out in a pure form.

The effect of the temperature of incubation is very marked. Incubation at 27°C. changes the pH of the medium from 6·8 to 8·4 in four weeks and titratable alkalinity increases from neutral to 25·0 cc. of N/10 per 100 cc., while incubation at 37·5°C. raises the pH to only 7·8 in four weeks and titratable alkalinity to 8·0 cc. N/10 per 100 cc. These differences are obviously due to much more profuse growth at 27°C. In one experiment the number of viable organisms after two weeks growth were 525 millions per cc. at 27°C. and only 56·5 million organisms per cc. at 37·5°C. There are considerable difficulties in obtaining exact counts owing to the clumping which occurs on prolonged incubation and an attempt is being made to solve this point by determining the weight of growth at the two temperatures.

2. *Normal Standards.*—Bloods from thirty normal adult Indians between the ages of 23–30 years were examined for chemical constituents ordinarily determined for chemical investigations. Our subjects were all medical students or interns, and weighed between 100 and 155 lbs. and measured five feet two inches to five feet ten inches. Half the subjects were on mixed diet, the other half were strict vegetarians.

Nitrogen and sugar determinations were carried out by the micro method of Folin (Jour. Biol. Chem. 88, 85, 1930), chlorides were estimated by Whitehorn method, cholesterol by Lieboff method, phosphorus by

Fishe and Subbarow method, serum calcium by Clarke and Collip method, and fibrin albumin and globulin by Wu method.

It is proposed to examine at least one hundred cases, but we give below the results obtained so far :—

Constituent.	Range.	Average.
Non-protein Nitrogen ..	15·18—21·92 mg. per cent. ..	18·94 mg. per cent.
Urea Nitrogen	7·96—14·53 ,, ,, ..	11·97 ,, ,,
Uric Acid	2·49—4·29 ,, ,, ..	3·15 ,, ,,
Creatinine	0·73—1·48 ,, ,, ..	1·23 ,, ,,
Total Creatinine	1·18—3·01 ,, ,, ..	2·26 ,, ,,
Amino acid Nitrogen ..	4·03—5·72 ,, ,, ..	4·86 ,, ,,
Chlorides as Sodium Chloride ..	339—500 ,, ,, ..	420 ,, ,,
Cholesterol	138—232 ,, ,, ..	184 ,, ,,
Inorganic Phosphorus ..	2·98—4·33 ,, ,, ..	3·67 ,, ,,
Serum Calcium (18 cases) ..	9·43—11·84 ,, ,, ..	10·50 ,, ,,
Fibrin	0·13—0·33 g. ,, ..	0·22 g. ,,
Albumin	3·77—5·01 ,, ,, ..	4·25 ,, ,,
Globulin	1·52—2·80 ,, ,, ..	2·11 ,, ,,

3. *Researches on Anti-plague Vaccine.*—See under Plague Researches.

Department of Public Health Chemistry and Bacteriology.

A. *Chemical Work.*

Waters.—During the year under report 38 samples of water were examined chemically of which 11 were examined for their mineral constituents as well. The sources from which they were obtained and the extremes of variation in total solids and chlorine are shown in the table. The samples are grouped into four divisions, viz. wells, treated waters, borings and surface waters, the last including rivers, tanks and tap supplies :—

Source.	Number.	Total solids.		Chlorides.		Potability.	
		Highest.	Lowest.	Highest.	Lowest.	Fit.	Unfit.
Wells ..	18	420·0	8·0	143·0	2·2	1	17
Treated waters ..	7	13·2	8·9	0·7	0·4	3	4
Borings ..	5	344·0	52·4	110·0	6·4	*Nil.*	5
Surface waters ..	8	33·6	10·8	3·2	0·3	4	4

It is significant that of the 18 samples of well water examined the only one found to be chemically fit for potable purposes was from a dhed-kui (i.e., a well probably exclusively used by the depressed classes).

Other works.—The department supplied stains and standard solutions to some departments of this Institute and various laboratories in the Presidency as usual.

B. Bacteriological Work.

22 samples of water were examined bacteriologically. Of these, 10 were samples of drinking water from various sources, and were examined with a view to determine their fitness for potable purposes. In addition 12 samples of swimming-bath water were examined, at the request of the Committee of the Breach Candy Baths. The examination consisted in determining (1) the number of viable bacteria per cubic centimetre of the sample aerobically on nutrient agar, and (2) the least quantity of the sample that was capable of producing acid and gas in MacConkey's neutral red bile salt lactose broth on two days' incubation at 37°C.

Drinking waters.—The sources from which the samples were derived are shown below :—

Surface waters	7
Wells	2
Boring	1

The bacteriological findings are shown in the following table :—

Total number of aerobic bacteria per cubic centimeter of sample.	Least quantity of sample fermenting MacConkey's broth.							Total number of samples.
	No fermentation in 100 cc.	100 cc.	10 cc.	1·0 cc.	0·1 cc.	0·01 cc.	0·001 cc.	
1—10	..	..	..	..	..	..	..	..
11—100	..	..	2	..	1	..	..	3
101—1000	..	..	..	3	1	..	..	4
1001—10000	..	..	1	..	1	..	1	3
Above 10000	..	..	..	..	..	..	..	..
Total number of samples 66.	..	..	3	3	3	..	1	10

From the above table it will be seen that none of the samples used for drinking purposes could be classed as bacteriologically fit for potable purposes when judged by the English standards.

ANTIRABIC DEPARTMENT.

During the year 7,059 persons underwent a complete course of antirabic treatment of whom 1,061 were treated at the Haffkine Institute and 5,998 at out-centres. The corresponding figures for the previous year were 736 and 5,006 respectively showing an increase of 1,317. There were 3 deaths from hydrophobia after treatment at the Institute and 40 deaths amongst those treated at out-centres giving respective mortalities of 0·28 per cent. and 0·66 per cent. and a combined mortality rate of 0·60 per cent.

Statistical returns.—The International Conference on Rabies held at Paris in 1927 considered that the estimation of the value of the treatment should be based on the receipt of health reports at six months after treatment which should amount to at least 80 per cent. of those treated. In those treated at the Institute 95·8 per cent. of health returns have been received which is highly satisfactory and for which we have to acknowledge the assistance of the Bombay Police in enabling the treated individuals to be traced. The position is less satisfactory in regard to those treated at out-centres, 73 per cent. of returns only having been received but is a slight improvement on the previous year and increased efforts have been made to enlist the help of local authorities in improving the returns.

Treatment at Institute.—1,692 persons applied for advice of treatment of whom 436 were advised that treatment was unnecessary. 1,256 persons commenced treatment of whom 1,061 completed a full course. Two persons showed symptoms of hydrophobia on arrival and are not shown in the tables. Two deaths from hydrophobia occurred within 15 days of completion of treatment and one case died more than 15 days after completion of treatment, giving a total mortality rate of 0·28 per cent.

Treatment at out-centres.—Treatment was given at 50 out-centres of which

14 were under the control of Civil Surgeons of the Bombay Presidency,
16 under Military,
5 under Civil Surgeons in U. P., and C. P.,
10 under Indian States,
4 under Railways,
1 under Portuguese Government.

5,998 persons received a full course of treatment at out-centres of whom six died of hydrophobia during treatment and are excluded from the statistics. 13 died within 15 days after completion of treatment and 27 died more than 15 days after completion. The mortality rate at the out-centres was 0·66 per cent.

Comparative results at Institute and out-centres.—The combined mortality rate for the year is the lowest on record since the antirabic

section was opened in 1922. It is not easy to assign the cause for the improvement, but this may in some degree be attributable to the use of the Paris strain of fixed virus which was introduced in March 1930. In that year no deaths occurred amongst those treated at the Institute, and in the present year only three deaths (0·28 per cent.). This should be compared with the results of the previous 8 years when the mortality at the Institute was always over 0·7 per cent. and on one occasion as high as 1·7 per cent. The difference between the results at the Institute and at out-centres may be attributable to the fact that the Institute cases mainly come from Bombay city itself, and are received usually on the day of the bite and submitted to a very thorough disinfection of the wounds. At out-centres they come at varying periods after the bite, and systematic disinfection with thorough opening up of wounds is less practised. Of the three deaths at the Institute two occurred in persons coming 16 and 45 days late respectively.

Paralytic accidents.—In the present year the first case of paralytic accident occurring after treatment at this Institute or its out-centres has been recorded, the total cases treated since the opening of the Antirabic Section being 44,187. The details of the case are as follows :—European lady, aged 29, unmarried, bitten on 7th June 1931 by a dog certified by a veterinary officer to be rabid, had a superficial tooth mark on arm and was also licked on abraded skin and was classified as Class II case; commenced treatment on 14th June 1931, and was given a 7 days course of 10 cc. of 2 per cent. carbolished vaccine daily. On 25th June 1931, two days after completion of treatment, she developed complete paralysis of lower limbs with paresis of both arms and mental torpor, retention of urine, and other signs of lower spinal paralysis. She remained in this condition for four months after which gradual improvement occurred, and at the end of a further two months was fully recovered and could walk without help. The vaccine of the same brew used for this case was given to other persons at the out-centre and no other case of this kind occurred. The vaccine was tested and found to contain the proper proportion of carbolic acid, and subdural inoculations into rabbits were without effect.

Training of medical officers in antirabic work.—Three medical officers, one deputed by the Surgeon General with the Government of Bombay, one from an Indian State, and one from the Military Department received training in antirabic work during the year. Further training of staff of hospitals at which out-centres exist is advisable as it appears that in many cases those carrying out treatment have insufficient knowledge of the methods of classification and of the proper courses of treatment which should be given.

Veterinary.—251 antirabic treatments were issued to the various veterinary surgeons for treating dogs and other animals. The brains of 67 animals were received from Bombay city and the adjoining suburbs. In 24 there was no evidence of rabies; and in 42 brains, Negri bodies were present. Brains of 110 dogs and other animals were received from various out-stations. 10 brains were unfit for examination; in 28

there was no evidence of rabies and 72 brains were found to contain Negri bodies.

Vaccine Department.

This department carried out the bottling and sterility testing of all plague vaccine and antirabic vaccine, and in addition manufactured small quantities of T.A.B., Cholera and other vaccine. Stock cultures of pathogenic organisms were maintained and issued to laboratories, etc. The department also had charge of poisonous snakes and undertook the collection of venom.

Plague vaccine—

4,182	brews of 1000 ccs. each were decanted.
145,316	bottles were filled with vaccine, labelled, numbered and sealed.
10,184	bottles were tested for sterility.
685,336	doses of the prophylactic were in all sent to the Despatch Department for packing.

Antirabic vaccine—

1,168	brews were decanted.
61,484	bottles were filled :
	1,721 bottles of 25 cc.
	31,640 ,, ,, 10 cc.
	28,123 ,, ,, 5 cc.
2,604	bottles were tested for sterility.
100,008	doses were sent to the Despatch Department

Other vaccines prepared—

4,115	ccs. Mixed T.A.B. Vaccine.
8,000	ccs. Cholera Vaccine.
740	ccs. Chicken Cholera Vaccine.

Killed Bacterial Emulsions prepared—

725	ccs. B. typhosus.
700	ccs. B. paratyphosus A.
1,150	ccs. B. paratyphosus B.
300	ccs. Coli communis C.
60	ccs. B. coli communis.

Stock Cultures—

385	various stock cultures were subcultured at least once a month.
13	special cultures of Pneumococcus were subcultured on special media every 4 days. (Blood medium was prepared from time to time for the above cultures.)
100	living cultures of pathogenic micro-organisms were issued.

Snakes and Snake Venom—

17 Cobras and 8 Russell's vipers were received during the year. On an average 8 cobras and 15 Russell's vipers were dealt with fortnightly for the extraction of venom. The following quantities of venom were issued chiefly to laboratories for experimental purposes :—

Cobra venom	..	..	30 grammes.
Russell's viper venom	..	..	30 ,,
Echis venom	..	..	10 ,,

Diagnostic Department.

(1) *By the General Diagnostic Department :*

Material examined.	Positive.	Negative.	Total.
A. *Blood*—			
(*a*) Films—microscopical examination for malaria..	3	40	43
for differential leucocyte count ..	..	..	18
from dog for piroplasma	..	1	1
(*b*) Count for R. B. Cs.	..	..	3
Count for W. B. Cs.	..	..	3
(*c*) Cultures for organisms (B. typhosus, etc.) ..	..	10	10
(*d*) Serological examinations—			
Wassermann reaction (including 42 of cerebro-spinal fluid).	1,491	1,485	2,976
Kahn test	877	1,443	2,320
Blood grouping	..	..	46
Agglutination tests—			
for Enteric group	37	67	104
for Undulant fever	..	6	6
for Dysentery	..	1	1
B. *Sputum*—			
Microscopical examination for *B. Tuberculosis* ..	11	13	24
Cultures for microorganisms	..	..	11
C. *Urine*—			
General analysis	..	..	64
Cultures	..	..	77
D. *Fæces*—			
(*a*) Microscopical examination for ova, cysts, amœbæ.	4	70	74
(*b*) Cultural examination for—			
miscellaneous organisms	..	..	29
cholera, typhoid, dysentery	6	23	29
(*c*) Chemical examination for occult blood ..	..	1	1
E. *Cerebrospinal fluid*—			
(*a*) Microscopical examination—			
for B. tuberculosis and other organisms ..	..	9	9
for Cell count	..	..	16
(*b*) Cultural examination	2	6	8

Material examined.	Positive.	Negative.	Total.
F. *Miscellaneous smear examinations*—			
Smear from purulent blister	..	1	1
Pus for *B. tuberculosis*	..	1	1
Smears for gonococci	2	11	13
„ leprosy	1	2	3
„ *B. diphtheriæ*	..	1	1
„ *B. pestis* (from rats)	..	11	11
„ Treponema Pallidum	..	1	1
Semen for spermatozoa	1	..	1
G. *Miscellaneous cultural examinations*—			
Swabs for *B. diphtheriæ*	..	4	4
Pus for pyogenic organisms	16	3	19
Nasal secretion	..	..	1
Swabs from nose and throat	..	..	2
Material from uterine cavity	..	1	1
Serum from blister	1	..	1
H. *Miscellaneous work*—			
Disinfectants for carbolic acid coefficient ..	..	..	9
Field dressing (for sterility)	..	..	1
Identification of cultures	..	..	15
„ snakes	..	..	3
„ Group serum supplied ..	..	..	2
Cultures supplied (*B. pestis*)	..	..	1
I. *Vaccines supplied*—			
(*a*) Autogenous vaccines	..	..	80
(*b*) Stock vaccines—			
Staphylococcus	..	..	108cc
Streptococcus	..	..	76cc.
Gonococcus	..	..	10cc.
Fowl cholera vaccine	..	..	110cc.

(2) *By the Biochemical Unit.*

The following analyses were done :—

	Number.
Blood—	
Non Protein Nitrogen	33
Urea Nitrogen	36
Uric Acid	33
Creatinine	26
Aminoacid Nitrogen	3
Chlorides	12
Cholesterol	28
Sugar	40
Phosphorus	2
Calcium	3
Fatty Acids	2
Fibrin	7
Albumin	8
Globulin	8
Hæmoglobin	33

	Number.
Blood—contd.	
Cell Volume	36
Indices	14
Van den Bergh	4
	328
Urine—	
General qualitative	62
Total Nitrogen	19
Urea	17
Uric Acid	10
Sugar	29
Chlorides	13
Phosphates	3
Albumin	31
Ammonia	1
Sulphates	2
Oxalate	1
Amino Acids	2
Urobilin	4
Acetone bodies	2
H ion concentration	1
Acidity	2
	199
Cerebrospinal Fluid—	
Protein	14
Globulin	5
Chloride	4
Sugar	4
Lange's Gold Test	10
	37
Miscellaneous—	
Glucose Tolerance test	7
Urea concentration test	4
Urine concentration test	4
Chylous fluids	2
Blood volume estimation	1
Basal metabolism	23
Gastric Analysis	10
	51
Total number of analyses ..	615

Pathological Section.

Table showing the total number of Pathological Tissues received and examined during the year 1931.

	Number
Malignant tumours	92
Non-malignant tumours	66
Miscellaneous pathological conditions	214
Tissues found to be normal	27
Tissues found to be unfit for examination ..	5
Total ..	404

Classification of Malignant Tumours and their situation.

	Carcinoma.	Sarcoma.	Epithelioma.	Endothelioma.	Rodent ulcer.	Total.	Remarks.
Lymphatic System—							
Axilla	3	..	..	..	..	3	
Groin	1	1	1	..	..	3	
Glands (Ingl.)	1	..	1	..	..	2	
Digestive System—							
Anus	1	..	..	..	..	1	
Rectum	1	..	..	..	..	1	
Tongue	..	..	1	..	..	1	
Urinary Organs—							
Bladder	1	..	..	..	..	1	
Male Reproductive Organs—							
Penis	..	..	4	..	..	4	
Prepuce	..	..	1	..	..	1	
Scrotum	1	1	2	..	..	4	
Testis	3	..	..	..	..	3	
Female Reproductive Organs—							
Breast	16	1	..	..	..	17	
Cervix	3	..	10	..	..	13	
Ovary	1	1	..	..	..	2	
Uterus	2	..	2	..	..	4	
Vulva	1	..	1	..	..	2	
Special Senses—							
Eye	1	3	..	..	..	4	
Orbit	1	..	..	..	..	1	

—	Carcinoma.	Sarcoma.	Epithelioma.	Endothelioma.	Rodent ulcer.	Total.	Remarks.
Skin and Subcutaneous Tissues.							
Abdomen	..	..	1	..	..	1	
Back	..	1	..	..	..	1	
Cheek	..	1	1	1	1	4	
Face	..	..	..	..	2	2	
Foot	..	1	2	..	..	3	
Leg	..	1	..	..	..	1	
Lip	..	..	1	..	..	1	
Thigh	..	1	1	..	..	2	
Connective Tissue and Muscles.							
Ear	..	..	..	1	..	1	
Neck	2	..	2	..	..	4	
Nose	..	1	..	..	..	1	
Palate	..	..	1	..	..	1	
Bones and Joints.							
Finger	..	..	..	1	..	1	
Jaw	..	1	1	..	..	2	
Total ..	39	14	33	3	3	92	

RESEARCH WORK.

Plague Enquiry.

(I) *Observations on the cultural characters of B. pestis.*

The methods of obtaining pure strains of *B. pestis* from passage animals and for testing purity and differentiating the contaminants which are occasionally found in heart blood have been the subject of further study. The heart blood of 554 passage animals was examined and the reactions of such contaminants as were found were tested. The methods of purity testing introduced by Dr. Gore were found to be satisfactory for the purpose; these tests consisting of (*a*) differential quantitative growth on blood agar and standard agar, (*b*) tests for production of (1) nitrate, (2) indol and (3) H_2S, (*c*) fermentation of glucose, mannite, laevulose, lactose, saccharose, and dulcite. Of these tests the most valuable has been that of comparative growth on blood agar and standard agar.

Amongst the organisms studied was *B. pseudotuberculosis rodentium* the differentiation of which from *B. pestis,* either by cultural methods or animal tests, has frequently presented considerable difficulty. The method of differential growth on blood agar and standard agar has been found to differentiate the two organisms with certainty.

The following table prepared by Dr. Gore shows the comparative reactions of certain organisms :—

Differentiation of B. pestis from certain Allied Bacilli.

Bacilli.		Differential colony growth on (1).			Protein cleavage Reactions (2).			Carbohydrate Fermentations (3).			
		Standard agar.	Blood agar.	Growth in fluid medium.	H_2S	Nitrite.	Indol.	Glucose.	Mannite.	Lactose.	Saccharose.
B. pestis	Virulent ..	No growth	Discrete colonies.	Floccular.	—	+	—	Acid	Acid	—	—
	Avirulent ..	Do. ..	Do. ..	Do. ..	—	+	—	Do...	Do...	—	—
B. pseudotuberculosis rodentium.	Virulent ..	Confluent or closely set colonies	Do. ..	Uniformly turbid.	—	+	—	Do...	Do...	—	—
	Avirulent ..	Do. ..	Do. ..	Do. ..	—	—	—	Do...	Do...	—	—
B. aviseptices.	Virulent ..	Numerous colonies.	Do. ..	Do. ..	—	+	+	Do...	Do...	—	Acid.
	Avirulent ..	Do. ..	Do. ..	Do. ..	—	—	+	Do...	Do...	—	Do.
B. enteritidis ..		Confluent colonies.	Do. ..	Do. ..	+	—	—	Acid and Gas.	Acid and Gas.	—	—

Notes.—(1) The differential colonial growth is ascertained by inoculating a blood agar slope with one-tenth the quantity of a high dilution of the culture under examination used for inoculating a standard agar slope. This is effected by well diluting a 1 mm. loopful of the culture (broth or peptone) in about 10 cc. of normal saline and inoculating a 1 mm. loopful of the dilution on a blood agar slope and a 5 mm. loopful on a standard agar slope.

(2) Protein cleavage reactions are determined by cultivation in peptone (Difco) water, and ascertaining the production of H_2S and Indole by Gore's 'Cotton Wool Plug' technique and of nitrite by his 'spot-plate' technique. The growth peculiarity noted by Dr. Petrie in broth culture may also be observed in the peptone culture.

After inoculation, all the media are incubated for two days at 37° C, and examined each day.

From the above table it will be seen that (1) Haffkine's qualitative surface growth character has been converted into a quantitative colonial character. Thus *B. pestis* does not show any growth on the standard agar though sown with about ten times the number of bacilli sown on blood agar which, on the other hand, shows numerous discrete minute colonies, not on one day's but on two days' incubation. This phenomenon stands in marked contrast with the confluent colonial growth of *pseudotuberculosis* on standard agar, and of fair sized discrete colonies on blood agar on 24 hours' incubation. This test brings out the fact that *B. pestis* requires blood or serum (human or rabbit) for its optimum surface growth while *B. pseudotuberculosis* does not, (2) Protein cleavage and (3) Carbohydrate reactions are practically the same. The uniform turbidity of growth of the peptone culture of *B. pseudotuberculosis* differs from the flocular character of the *B. pestis* peptone culture.

The characters that help in the differentiation of the other bacilli from *B. pestis* being evident under all the three groups of characters, have not been specially emphasised here.

(II) *Researches on anti-plague vaccine.*

(*a*) *A biological method for the standardisation of anti-plague vaccine.*—In the Annual Reports for 1929 and 1930 the progress of an investigation into experimental methods suitable for testing the immunising value of the Plague Vaccine has been detailed. This work has aimed at obtaining a technique which will enable successive experiments and tests to be directly

comparable and involves (*i*) the use of a measured test dose of *B. pestis* of regular and high virulence and (*ii*) the use of an experimental animal of regular high susceptibility. Major Sokhey and Dr. Maurice have continued their work on this subject with the object of eliminating certain of the difficulties which have been experienced in regard to obtaining absolute regularity in the test dose. The main difficulty which has been experienced is the occasional loss of virulence of the passage strain of *B. pestis* when maintained in trapped *R. rattus*, this being specially liable to occur during the hot weather. The loss in virulence is only a relative one and is detected as the result of the use of accurately measured infecting doses of *B. pestis* amounting as a rule to less than 100 organisms which will normally kill 100 per cent. control rats or mice. It has been found that 500 or 1,000 organisms of a particular passage may be necessary to kill, but such a variation would probably pass undetected by the methods formerly used in preparing, infecting material for tests when accurate counting was not employed and the check on virulence which is now possible enables only the most virulent material to be employed for the manufacture of vaccine. The results of the recent work have shown that the albino mouse is suitable for the maintenance of cutaneous passages and that the changes in virulence experienced when *R. rattus* is employed are less likely to occur if it is used. White mice have been taken into use for all experimental work on the immunising value of the vaccine and for comparative tests of the value of the various procedures employed in the preparation of the plague vaccine.

The method of testing has now been standardised and has given full agreement between duplicate determinations. The details of the method are as follows :—

Animals.—Laboratory mice over 3 months old, weighing between 20 g. and 30 g. 5 mice are used for each dose of the vaccine to be tested.

Dose of vaccine.—Every brew of Haffkine's anti-plague vaccine tested during the year has given complete protection with 0·03 cc. of vaccine per animal, and in the case of vaccines tested immediately after they were made, as little as 0·007 cc. gave complete protection. So, for each brew to be tested, three doses are selected 0·03 cc., 0·015 cc., and 0·007 cc. Dilutions of the vaccine in broth are so made that the quantity actually to be injected in each case amounts to 0·5 cc. per animal. For each dose five animals are used.

Interval between the giving of vaccine and the giving of test dose of plague. 10 days.

Test Dose.—0·5 cc. of a 1 : 500,000 dilution of a 48 hour broth culture of a strain of *B. pestis* of a known virulence grown at 27° C. This amount of test dose contains between 30 and 120 organisms—most often about 100 organisms. The strain of *B. pestis* selected for the purpose is such that 0·5 cc. of 1 in 5 million dilution, containing 3-12 organisms, per animal kills all the 5 mice usually injected. It is seldom that an animal escapes. This strain is kept up by passage in the mouse,

The test dose is made as follows :—A mouse is inoculated by the cutaneous route; a patch on the abdomen is shaved so that a little serum oozes, and a little of the spleen of a mouse just died of plague is rubbed in. The animal thus inoculated dies in 3 to 4 days and a little of its heart blood (a fraction of a drop) is taken up in a pipette and is added to 1 cc. of citrated saline. 1 mm. loopful of this dilution is added to 5 mm. loopful of saline and thus four serial dilutions are made, and 1 mm. loopful of each dilution is planted on to a blood agar slope. The slopes are then incubated for 5 days at 27° C. On the sixth day a slope showing isolated colonies is taken and a single colony is lifted off the slope and is broken up in 10 cc. of broth. This is incubated at 27° C. for 48 hours, then 0·5 cc. of this culture is added to 10 cc. of broth and incubated at 27° C. for 48 hours. Dilutions of this second subculture in broth are made, and 0·5 cc. of 1 in 500,000 dilution is used as test dose for each mouse.

Duration of test.—Animals are observed for 30 days after the test dose is given. Any animals dying during that period are dissected and examined for evidence of plague ; all those alive at the end of the period are killed and examined.

Controls.—For every test five animals are used as controls and are given only the test dose of plague and no vaccine. All the controls die in 3 to 7 days.

Evaluation of results.—The smallest dose of the vaccine which saves at least 4 out of 5 mice given that dose is taken as the protective dose for comparison with other vaccines. Duplicate tests should agree.

Using the experimental method outlined above the following comparative results have been obtained with different anti-plague vaccines :—

———	Human dose recommended by makers.	Minimum dose required to protect mice.	Remark.
1. Haffkine Anti-plague vaccine, Brew, 8329.	3–4 cc.	0·007 cc.	
2. Institute Pasteur, Paris, Vaccine anti-pest, Batch 1, P.	1–8 cc.	..*	*As large a dose as 1 cc. failed to protect mice, a larger dose could not be given conveniently.
3. Bayer, Meister Lucius, Anti-plague, vaccine Control R. F.	1·0 cc.	1·0 cc.	
4. Swiss Serum and Vaccine Institute, Bern. Lustigs Anti-plague (especially made for the test).	1·0 cc.	0·1 cc.	
5. Lister Institute, London, Anti-plague vaccine, Batch 91.	1·5 cc.†	0·007 cc.†	†A dose smaller than this has not been tried yet.

The Lister Institute vaccine which has shown as good results as the Haffkine vaccine has a high bacterial content and a higher opacity than the other vaccines. The supernatant fluid of an equal quantity of the Haffkine vaccine showed protective value equal to the whole vaccine.

(*c*) *Effect on the virulence of B. pestis of growth on different media at* 27° *C. and* 37·5° *C.*—A culture of *B. pestis* of known virulence (i.e. 3-10 organisms of which killed 100 per cent. of the animals used) was taken and weekly subcultures were made on Nutrient Agar, Blood Agar and Nutrient Broth. One subculture on each medium was kept at 27° C. and one at 37·5° C. Virulence tests were made approximately every four weeks.

The effect of the higher temperature, 37·5° C. was found to be most profound; virulence was lost very rapidly on all the three media; the loss of virulence being most marked in nutrient agar subcultures. Nutrient agar—4th subculture, 31 days, 1,010 organisms, failed to kill any animals; Blood agar—16 subculture, 112 days, 144,500 organisms failed to kill; while Nutrient Broth—16th subculture, 116 days, 75,000 organisms killed four out of five animals injected.

At 27° C. the loss of virulence was less rapid, but the loss again was in the same order, being most marked on nutrient agar cultures than on blood agar cultures, and least in broth cultures. Nutrient agar—14th subculture, 102 days, 500 organisms failed to kill; blood agar—16th subculture, 121 organisms killed two out of five animals; while broth—21st subculture, 153 days, 49 organisms killed four out of five animals.

(*d*) *Relationship of the virulence of B. pestis to the immunising value of the resultant vaccine.*—The preliminary experiments show that virulence and immunising value go hand in hand. Vaccines made from strains of *B. pestis* made a-virulent have not protected the test animals, while the same strains when used in highly virulent state afforded protection.

(*e*) *Relationship of the virulence to the toxicity of the B. pestis.*—Our work shows that virulent strains are much more toxic than the same strains made a-virulent. In one experiment 200 million killed virulent organisms per mice killed all the test animals, while 3,000 million killed or live organisms of the same strain rendered a-virulent produced no deaths in these animals.

(*f*) The investigations into the changes occurring in the vaccine during incubation and the effect of temperature of incubation on growth have been mentioned under the Biochemical Section.

(*III*) *Epidemiology of Plague.*

An enquiry into the comparative immunity to plague of rats from different parts of India was sanctioned in July 1931 and work on the subject was commenced by Rao Bahadur G. D. Chitre in August. The Plague Research Commission first showed in 1911 that while rats from Madras City which had been free from epidemic and epizootic plague were highly susceptible to a small infecting dose of *B. pestis* rats from

other towns such as Bombay and Poona in which severe epidemics had occurred for a number of years would only show a comparatively small mortality from the same dose. During the last 20 years Madras City rats have been constantly employed for experimental work at the Institute and they have been found to maintain their susceptibility. Occasional comparative tests have been made with rats from other places which have suffered from plague in varying degree and their resistance to infection has been found to be roughly correlated with the incidence of plague which has occurred over a period of years. The development of immunity in rat populations after a period of epizootic plague which is indicated by these findings suggests that this may be a controlling factor in the rise and fall of epidemic waves of plague and its estimation may give a guide to the likely progress of the disease in future. In the case of Bombay City epizootic plague has never been absent since the first introduction of the disease in 1896 and we continue to observe the epizootic day by day. The epizootic is now at a very low level but is continuous and the human plague cases only amount to about 20 yearly. It is probable that the low level of the epizootic and consequent human cases is dependent on the comparative resistance of the rats to plague infection. In the tests of comparative immunity which were formerly carried out with rats from different towns an infecting test dose of *B. pestis* was used which could not be accurately estimated either in regard to the number of organisms given or in regard to their virulence. Successive tests could only be of limited value for comparison with each other and when the present work was undertaken with the purpose of watching over a period of years any increase or decrease in immunity of the rat population it was essential to utilise an experimental technique, by which a definite standard of infection could be reproduced as required. The technique which had been evolved for the testing of the immunising value of the plague vaccine provided a suitable method for the purpose and a standard dose of *B. pestis* culture dilution with a known range of organisms was employed for the purpose.

A first point of importance in regard to the testing of immunity is the question of dosage of the organism to be used. The following figures will illustrate this :—

Name of place	*B. pestis* colonies inoculated per rat	Number of rats inoculated	Deaths from acute plague amongst those inoculated	
			Number	Per cent.
Bombay city	64	22	0	0·0
Madras city	64	4	3	75·0
Bombay city	64,500	25	1	4·0
Madras city	64,500	4	4	100·0
Bombay city	32,250,000	25	25	100·0
Madras city	32,250,000	4	4	100·0

It will be seen that a high degree of immunity can be demonstrated, against a small dose of *B. pestis* but if a sufficiently large dose is used resistance will be completely overcome.

With a dosage selected on the basis of these results six successive experiments were carried out up to the end of the year with the following results :—

Name of city or town	Aggregate plague death rate per mille 1899 to 1929 (inclusive)	Number observed during 21 days	Death from acute plague	Percentage deaths from acute plague	Percentage survivors
Bombay city ..	199·88	83	5	6·0	94·0
Ahmednagar city ..	209·28	47	11	23·4	74·5
Madras city ..	0·396	10	8	80·0	20·0
Surat city	101·11	45	9	20·0	80·0
Madras city ..	0·396	14	12	85·7	14·3
Nasik city	363·43	50	0	0·0	100·0
Madras city ..	0·396	10	9	90·0	10·0
Sholapur city ..	272·37	34	2	5·9	94·1
Dhulia city ..	206·32	39	8	20·5	79·5
Madras city ..	0·396	20	20	100·0	0·0
Ahmedabad city ..	56·53	45	7	15·6	84·4
Madras city ..	0·396	10	10	100·0	0·0
Bombay city ..	199·88	10	0	0·0	100·0
Karachi city ..	315·54	35	6	17·1	82·9
Madras city ..	0·396	10	10	100·0	0·0
Bombay city ..	199·88	10	0	0·0	100·0

The combined results (averaged) in relation to the previous incidence of plague are shown below :—

Name of city or town	Aggregate human death rate per mille 1899 to 1929 (inclusive)	Percentage deaths from acute plague in inoculated rats
Madras city	0·396	93·2
Ahmedabad city	56·53	15·6
Surat city	101·11	20·0
Bombay city	199·88	4·9
Dhulia city	206·32	20·5
Ahmednagar city	209·28	23·4
Sholapur city	272·37	5·9
Karachi city	315·54	17·1
Nasik city	363·43	0·0

The nature of the problem makes it unlikely that an exact mathematical correlation will be found between the incidence of plague based on average

figures over a prolonged period and the degree of resistance of rats to infection. The continuity of an epizootic is likely to be an important factor and the high degree of immunity of the Bombay rats supports this possibility. In the case of rats showing a considerable degree of resistance of infection a certain percentage examined 21 days after infection showed necrotic and suppurating lesions which indicated that they were recovering from an attack of plague.

The work is being extended to a large number of towns in the Bombay Presidency and also to the Punjab and other provinces and should provide valuable information as to the likelihood of future development in regard to plague in India.

(*IV*) *Anti-Plague Serum.*

Dr. Naidu and his assistants continued their work on the preparation of a therapeutic serum for plague during the year. At the beginning of the period three buffaloes, two bullocks and four sheep were under immunisation and to this number then was added six calves at a later period. Successive bleedings were carried out followed by re-immunisation on the same lines as before and a total of twenty-five litres of serum was prepared. A series of trials of the serum on 76 cases and controls was made at Hyderabad with highly satisfactory results, which, on account of the interest shown in the matter were detailed in the report for 1930 of which publication had been delayed. An attempt was also made to carry out serum treatment at Chaprar in the Punjab but it was found that under village conditions there was the greatest difficulty in obtaining cases for treatment and in the absence of hospital facilities satisfactory observation with bacteriological control was not attainable. A small supply of serum was sent to the Mysore State authorities for trial at Bangalore but only four cases were treated with inconclusive results all being of the severe septicaemic type. The Dharwar District in which plague was reported to be epidemic was also visited but with the diminished incidence of plague and the scattered distribution of cases no satisfactory trial could be obtained. It is obvious that under the present conditions considerable difficulty will be likely to be experienced in obtaining cases suitable for treatment under hospital conditions but all Directors of Public Health have been circularised in order to obtain early reports of outbreaks which may furnish an opportunity of a satisfactory trial.

Potency tests of the serum.—Potency tests done in rabbits showed that most of the sera prepared by the present method were of medium or good quality, saving 60 to 100 per cent. of the experimentally infected animals. The infecting dose at first used was 0·0003 mgm. of spleen of a plague passage rat. Later, a standard culture virus was given in a dose of 1 cc. of broth culture containing between 4,000 and 10,000 *B. pestis*. The infecting dose was given subcutaneously, and the serum intravenously, the first dose of 1 cc. being administered 24 or 48 hours after infection and a similar dose repeated on two subsequent days.

Other experiments are in progress as to the protective value of single dose treatments, and with larger single doses recovery has been obtained. It is probable that protection by single measured doses will be capable of forming a basis of control of the process of immunisation and may be of use in standardisation.

The suitability of white mice for the standardisation of the serum is being studied and it appears likely that these animals may be found useful for the purpose.

The results with rabbits are somewhat irregular and these animals are very variable in their response to the infecting dose of *B. pestis* used. A comparison of the value of the sera of different animals immunised has thus been made somewhat difficult and it has not yet been determined which animal should be finally selected for the production of the serum on a large scale.

Properties of the serum.—A technique has been evolved which demonstrates the bactericidal value of the serum and which may be applicable to assist in standardisation. Major S. D. S. Greval, I.M.S., has worked out a complement fixation technique which gives satisfactory and regular results. Complement fixation appears to proceed parallel with the bactericidal property of the serum.

Concentration of the serum.—Used in the large doses which appear to be necessary the serum tends to have a cardio-depressant effect in the serious septicaemic type of plague case. The removal of protein fractions other than those with which the causative value of the serum is associated is undoubtedly called for, and in November Dr. Naidu proceeded on leave to Europe to study serum concentration methods at the principal Serum Institutes in England and the Continent with the object of determining which method will be the most suitable for use with the anti-plague serum. The serum from several of the animals under immunisation was forwarded to Dr. Naidu and the concentrated fractions returned to this Institute for test. The results have been highly satisfactory, concentrates of the eglobulin and pseudo-globulin fractions proving equal in value to the whole serum. A further study of the fractions with suitable experimental animals should enable outstanding points to be determined and should enable a non-toxic serum of satisfactory potency to be produced.

(*V*) *Chemotherapy of Plague.*

The experimental work on the treatment of plague with the series of twelve halogenomercuriphenols which had been prepared was continued during the year. The experimental animals used for the tests was the pure strain of blue rabbits bred from imported English stock, which had been considered, as the result of the previous years work, to be likely to give the most consistent results. It was unfortunately found that these animals bred very slowly in the Bombay climate, giving small litters and showing a considerable degree of loss during the first four weeks of life. The small number of animals available seriously interfered with the

progress of the work and the use of small batches for each experiment resulted in the results obtained being unconvincing and liable to error. In the previous year the work which had been carried out by the subcutaneous route showed the relative toxicity of the compounds and indicated that this was not proportionate to the mercury content. It had also showed that divided doses were better tolerated and more likely to be of use in treatment than single maximum doses. The work was repeated in the present year by the intravenous route, a determination of the safe dose of each drug by this method being first carried out. Of the twelve compounds two were rejected as being highly unstable chemically. Systematic trials of the remaining drugs were carried out on the rabbits, a measured infecting dose of *B. pestis* being given and the selected drug administered intravenously at various intervals after infection. Dosage and intervals were varied according to the results of successive experiments. The general indication from the results obtained was that, while some degree of prolongation of life was observed in certain cases and possibly a diminution in septicaemia after treatment, no definite evidence of therapeutic value of the compounds could be claimed. In the course of the work a study was made of the pathological conditions in the kidney and other organs which might be attributable to the use of the compounds. An attempt was made to extend the basis of the tests by the use of susceptible *R. rattus* and laboratory bred albino mice which would be available on a much larger scale but it was found that after a single injection into the vein of the tail used for this purpose the resulting damage and thrombos is made it impossible to continue the treatment.

Of the compounds tested difluoromercuriphenol appeared to give the best results and its intravenous use was to a certain extent encouraging.

Tuberculosis Inquiry.

The Inquiry was continued by Dr. Soparkar and his assistants at the Haffkine Institute, Bombay, and the following note on the work has been submitted :—

"Study of the character of bacilli isolated from cases of human tuberculosis was continued and sixteen further strains were investigated. Seven of these were isolated from adults and nine from children under fifteen years of age. Of these 12 were cases of glandular tuberculosis and two of bone and two of joint tuberculosis. All except one strain showed the characters of the human type.

This strain was found to differ from the usual human type of tubercle bacillus and showed variations in morphology, cultural characters and pathogenicity. The strain was isolated from scrapings of a tuberculous sinus about the hip joint of a man aged 42. The first guineapig inoculated with the material failed to develop tuberculosis, but when passage was made into another guineapig a slight local lesion developed from which the strain was isolated. This is a second instance in which a strain of

very low virulence has been isolated from a case of human tuberculosis, the first one being from a female breast.

Filterability of the tubercle bacillus.—Work on the question of the existence of a filterable virus in tuberculosis has been in progress for some time. It was stated in the report for the previous year that in five out of ten experiments strains of tubercle bacilli were isolated from animals inoculated with the filtrate of tuberculous sputa and pathological material obtained from animals. In one more case recently a strain was isolated from the animal inoculated with the filtrate but unfortunately it was lost through an accident. In the remaining four experiments although acid fast bacilli were detected in more than one passage animal no cultures could be isolated as the lesions were contaminated.

Of the cases from which strains were isolated the material in three instances was obtained from human subjects and in two from animals inoculated with bovine strains. These strains were further studied in order to establish their identity with the strains from which the filtrates were obtained. The strains obtained from human cases showed characters of the human type. One of the strains, however, appeared to possess powers which produced extensive disease of the joints and in some respects resembled an avian culture. Another strain showed evidence of dissociation and two types of colonies, rough and smooth, were found in the culture. These were separated and animal tests are being made for further study.

The other two strains which were derived originally from bovine bacilli showed unusual characters. In one case the source of the filtrate was tissues of a guineapig inoculated with an English bovine strain. The filtrate was passed through animals and a strain was isolated after the seventh passage. This was tested on animals but the virulence was found to be much lower than that of the original strain. Calmette and others who claim to have proved the existence of a filter passing form of the tubercle bacillus have found that these filterable elements show attenuated virulence and the results obtained in our experiments appear to corroborate these findings.

In another case tissues of a guineapig that had died after infection with a bovine strain of Indian origin were used for filtration. The filtrate was inoculated into another guineapig and successive passages were made in series and a culture was eventually isolated after the sixth passage. Cultural characters of the strain appeared different from those of the strains originally employed. Virulence tests were made upon rabbits, guineapigs and fowls and it was found that the strain was pathogenic to rabbits and fowls but not to guineapigs, indicating that it differed in characters from the mammalian type to which the culture originally belonged and showed those of the avian type. Further experiments are in progress to see if the original strain which was obtained from a samber from the local Zoological Gardens was in reality a mixture of bovine and avian strains of which only the avian passed through the filter or whether there was any possibility of transformation of one type into another.

Having obtained positive results in six out of ten experiments when several other workers, viz. Cooper and Petroff, Gloyne Glover and Griffith, Alston and others, had obtained entirely negative results, it was necessary to determine whether the positive results obtained in the guineapigs employed in the filtration experiments described above were due to any spontaneous infection caught by these animals in the laboratory. For this purpose control experiments were made in which twelve guineapigs were maintained under conditions identical with those in which the guineapigs of the previous filtration experiments were kept. They were killed at regular intervals and serial inoculations were made from guineapig to guineapig until nine passages were made as in the case of the original experiments involving the use of 117 guineapigs and the control guineapigs were treated in all respects in the same manner as were previous experimental guineapigs except that they were not inoculated with any filtrate. All the guineapigs were carefully examined post-mortem and the tissues subjected to microscopic examination. In only five instances acid fast bacilli were detected but when cultures were attempted on 65 occasions from the tissues of several guineapigs including those showing acid fast bacilli in no instance was any growth obtained and the results were entirely negative. This would indicate that the positive results obtained in the previous filtration experiments could, in all probability, not have been due to any spontaneous infection of the experimental guineapigs. Although no definite conclusions can be drawn at this stage the results obtained so far go to support the view of French workers of the existence of a filter passing stage of the tubercle bacillus.

Spontaneous Tuberculosis among Laboratory Animals.—Some experiments were made during the previous year on this subject of practical importance. The work was continued during the year under review and two further series of experiments were made, one with guineapigs and the other with rabbits.

The experiments made on guineapigs have been described above. Their results have been negative and they are in agreement with the general trend of opinion.

Experiments on Rabbits.—In this series 14 rabbits were placed in cages adjoining to those containing infected ones. Ten of these had an infected neighbour on one side only and four had them on either side. The infected rabbits were those inoculated with a virulent strain of bovine bacilli. This had produced affection of the lungs in every case and had thus furnished sufficient chances of infection. The condition of the lungs of these rabbits was confirmed by post-mortem examination and as soon as an infected animal died it was replaced by another to ensure continuity of exposure.

The total period of exposure to infection lasted in eight instances from 118 to 147 days and in six instances from 211 to 226 days. After this period the animals were allowed to remain in a separate place for about 3 to 5 months in order to allow time for any infection they might have caught during the last days of exposure to develop. At the end of this period of observation the animals were destroyed and subjected to a

careful post-mortem examination. Only one out of 14 animals showed lesions of tuberculosis in the lungs and kidneys. In this animal the exposure had lasted 142 days. The rest of the animals, some with even much longer exposure on one or both sides, failed to show any evidence of infection.

These largely negative results obtained under conditions specially designed to facilitate infection indicate that spontaneous tuberculosis in these animals under laboratory conditions obtaining here is a matter of rare occurrence.

Epidemiology of Tuberculosis.—A systematic survey of the prevalence of tuberculous infection among various groups of the population was commenced in November 1930. The term tuberculous infection includes latent infection in individuals apparently healthy and should be distinguished from tuberculous disease. The presence of tuberculous infection is detected by the application of certain tests and a positive reaction is an indication of tuberculous infection but not necessarily of clinical disease.

Mention was made in the report for previous year of the tests having been carried out in some of the Jails of the Bombay Presidency and on the operatives of the textile mills at Ahmedabad and in a few other places. The method employed was the cutaneous tuberculin test (von Pirquet) and observations were made at the end of 48, 72 and in many cases 96 hours. The survey was continued during the year under report and through the courtesy of the Inspector General of Prisons, Madras, inmates of most of the Jails of the Presidency were examined (Salem 1,387, Coimbatore 768, Cannanore 726, Madura 384, Trichinopoly 763, Madras 742, Vellore 948, Presidency Jail for Women, Vellore, 237, and Bellary 697).

Examination was also carried out in Borstal and Certified Schools and in the Jails of the adjoining Indian States. (Palamcottah 513, Tanjore 349, Chingleput 318, Bellary 81, Bangalore 526, Trichur 128 and Trivandrum 454.)

In the Indian States of Mysore and Travancore the survey was also carried out among the Police and the Military forces for purposes of comparison (Police, Bangalore, 379, Nair Brigade, Trivandrum, 757).

Tests were also carried out on the operatives of the Mills of the Presidency and the inmates of hospitals and staff of the M.S.M. Railway Audit Office. (Kaleswaram Mills, Coimbatore, 423 ; Sitaram Spinning and Weaving Mills, Trichur, 666 ; Mental and Ophthalmic Hospitals, Bangalore, 178 ; Railway Audit Office, Madras, 563.)

The school going population of several towns in the Presidency was also tested. (Cannanore 752, Palamcottah 231, Trivandrum 2,945, Nagercoil 1,470, Madura 2,253, Trichinopoly 1,525, Chingleput 524 and Bellary 690).

In the Bombay Presidency, children in schools at Karachi (1,760) and at Hyderabad, Sind, (9,272) were also tested.

Altogether over 50,000 persons belonging to different groups of population in portions of the Bombay and Madras Presidencies have been tested.

A few general observations may be made on the results of tests so far carried out.

In Jails in the Bombay Presidency Hindus showed a higher proportion of reactors to the test (52·41 per cent. among 3623) than Muhammadans (39·16 per cent. among 3539) while the contrary was the case in the Madras Presidency (45·61 per cent. among 7356 Hindus and 59·54 per cent. among 1082 Muhammadans).

The proportion of reactors varied in different Jails. The percentage varied in the Bombay Presidency from 25·06 in Sind Convict Gang, Hyderabad, Sind, to 71·15 per cent. in Byculla Jail, Bombay, and in the Madras Presidency from 33·33 per cent. in District Jail, Madura, to 61·34 per cent. in the Central Jail, Cannanore.

In contrast to Jails in the Bombay Presidency the proportion of positives among Muhammadan operatives in the textile mills was higher (74.15 per cent. among 708) than among Hindus (67·81 per cent. among 6513). In the Madras Presidency the proportion of reactors among Hindus in the textile mills was lower (42·94 per cent.) than in the Bombay Presidency.

While the regular police force in Bangalore and the Nair Brigade in Trivandrum showed a comparatively high proportion of reactors, their families showed a very low proportion, particularly so the children.

	Number tested.	Number positive.	Percentage positive.
			per cent.
Police, Bangalore, adults	204	115	56·37
Police, Women and children	175	41	23·42
Military (Nair Brigade)	595	261	43·86
Military, Women and children	172	13	7·55
Military, children alone	100	1	1·00

Schools.—In schools in Sind generally the proportion among Hindu males was lower (28·98 per cent. among 6376) than among Muhammadan males (38·99 per cent. among 1731). The proportion among girls was lower than among boys.

In the Madras Presidency a similar proportion was noted among both communities (28·63 per cent. among 7544 Hindus and 41·82 per cent. among 263 Muhammadans). The proportion of reactors among Christian girls, however, was very low (13·34 per cent. among 1100). In Nagercoil the proportion of positives among girls in the Mission School was the lowest observed. Out of 486 girls tested only 28 or 5·76 per cent. reacted.

The significance of the figures will be seen from comparison of data available for other countries.

For example in Germany out of 12,304 children tested over 51 per cent. reacted.

In France the proportion of positive reactors to Pirquet test was 55 per cent. among 1226 children tested.

In England out of 1000 children under the age of 14 examined 56 per cent. reacted.

In New York City out of 1695 children tested 64 per cent. showed a positive reaction.

In Philadelphia the proportior among 4107 children between 3 and 20 was 74 per cent.

It will thus be observed that School children in India show a comparatively much smaller proportion of reactors than children in many of the Western countries."

Minor Enquiries.

(a) *Sprue Enquiry.*

Dr. Bilimoria and Dr. Walwyn studied the histological appearances in the intestinal tract under different conditions to determine the value of the previous findings in regard to the intestines in sprue cases. Examinations were made in the case of 24 patients dying from diseases other than sprue, tissues being fixed at intervals of from two to twenty-seven hours after death. The degenerative changes in the intestine were not regularly progressive or in direct relationship to the period elapsing between death and the fixation of the material. In cases of prolonged illness, e.g. nephritis and heart disease, degenerative changes were more marked than in accident cases dying soon after admission to hospital. The ilium was always found to be thinner than any other part of the intestinal wall. In the caecum changes set in early and did not progress rapidly. The changes in the duodenum were more advanced than in the stomach. Complete erosion of the mucous membrane with exposure of the submucous layer was never seen.

Tissues from two fatal accident cases fixed at one and two hours after death were kindly supplied by the Pathologist, Rangoon General Hospital and provided useful normal material, the only defect seen at most levels being separation of the surface epithelium mainly due to the technique of preparation and sectioning. No post-mortems in sprue cases were obtained, a fact probably attributable to the successful results of liver treatment which is now largely used. The results will be dealt with by Colonel F. P. Mackie in relation to his previous work on Sprue. This enquiry has now been terminated.

(b) *B. avisepticus Enquiry.*

The work which has been done at this Institute by Anderson and Wagle is a continuation of that done by Anderson, Coombes and Mallick previously done at the Central Research Institute, Kasauli. Cultures of

the Smooth and the Rough variants of *B. avisepticus* previously isolated by the above mentioned workers were obtained from Kasauli where they had been maintained on blood agar and Douglas agar respectively.

Having determined their purity by studying their cultural characters, morphology and virulence, sera were prepared from each type in rabbits. For the first few injections formalin-killed saline suspensions were given subcutaneously, and these were followed by giving live saline suspensions intravenously in gradually increasing doses. The injections were given at weekly intervals and rabbits were bled when further injections failed to raise the titre. During the course of immunisation, the virulence of both type cultures was repeatedly checked by determining their M.L.D. for pigeons and the identity of the organisms was controlled by protein and sugar reactions and the agglutination tests. In the case of the Smooth-type cultures, the virulence had to be kept up by frequent passage through pigeons.

Serological Tests and their Results.—The sera, so prepared, have been studied employing the following methods :—(1) Cross-agglutination and Cross-absorptions tests, (2) Precipitation tests with a soluble antigen prepared by Lancefeld's method.

A large number of cross-agglutination and cross-absorption tests, as well as the precipitation tests with the prepared antigen have so far failed to show any appreciable differences between the two types except that throughout the tests, generally speaking, the rough type is the better agglutinator. Owing to the constant tendency of the two types to revert to a common intermediate type, and the great difficulty we have experienced in maintaining the two variants unchanged, it seems likely that our sera were not sufficiently specific and fresh sera were prepared in which it was hoped that these fallacies would be eliminated. But the work had to be stopped owing to the transfer of Lt.-Col. Anderson to Rangoon and that of Dr. Wagle to Plague Inquiry at this Institute.

Acid and Salt Agglutination.—Acid agglutination has revealed definite differences between the two types.

Salt agglutination, although the results were in some respects rather irregular, has also shown the greater agglutinability of the rough variant, while the smooth type has always remained stable.

(c) *Leptospiral Infection of Bombay City Rats.*

The incidence of leptospiral infection of wild rats caught in the different wards of Bombay was investigated by Anderson and Wagle.

Three hundred rats in all were examined, 100 each of the three common species, *Rattus rattus*, *R. norvegicus* and *Gunomys Sp.*

The rats were examined for kidney infection using the technique of Taylor and Goyle as given in their memoir on Leptospirosis in the Andamans. Cultures in Fletcher's Medium were made as required from those specimens which were positive by dark-ground examination.

Of the total number of rats examined 39 or 13 per cent. were found to harbour leptospira in their kidneys.

The distribution among the three species was, however, very unequal and was as follows; *R. rattus*, nil infected; *R. Norvegicus*, 32 per cent. infected; *Gunomys Sp.* 7 per cent. infected.

The infected rats came from every part of Bombay, none of the wards being free. There did not appear to be any difference in the degree of infection in different parts of the City.

For some reason the house rat does not appear to have acquired the infection.

A few preliminary tests showed that the two strains isolated from the two species of rat were serologically identical. They differed serologically, however, from two human strains obtained from the Andamans, and from a water stain.

The research work carried out on (*a*) Plague Vaccine (*b*) Epidemiology of Plague, (*c*) Chemotherapy of Plague, (*d*) Anti-plague Serum, (*e*) Tuberculosis, and also the subsiding work in connection with the Sprue Enquiry, the *B. avisepticus* Enquiry and the work on a reduced scale in connection with Maternal Mortality and Morbidity mentioned in the General Review was generously financed by the Indian Research Fund Association which also provided personnel for these various investigations. Without the assistance of the Association the potentialities of the Institute as a centre of research would be largely lost.

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APPENDIX

Statistics of Antirabic Inoculation.

(Standard Tables approved for use in India.)

APPENDIX.

TABLE I.

A general statement regarding patients treated at the Institute and its Centres from 1922 *to* 1931.

Year		Europeans				Asiatics				Europeans and Asiatics combined			
		Total treated	Total deaths	Total death rate per cent.	Advice cases	Total treated	Total deaths	Total death rate per cent.	Advice cases	Total treated	Total deaths	Total death rate per cent.	Advice cases
1922	Institute	64	..	..	..	365	4	1·04	175	429	4	0·89	175
	Centres	7	..	..	..	236	..	..	..	243	..	..	..
	Combined	71	..	..	..	601	4	0·66	175	672	4	0·61	175
1923	Institute	115	..	..	59	730	10	1·37	359	845	10	1·18	418
	Centres	38	..	..	..	1,521	12	0·78	260	1,559	12	0·76	260
	Combined	153	..	..	59	2,251	22	0·97	619	2,404	22	0·91	678
1924	Institute	62	1	1·61	41	525	9	1·71	372	587	10	1·70	413
	Centres	85	..	..	..	2,880	28	0·97	330	2,965	28	0·94	330
	Combined	147	..	..	41	3,405	37	1·08	702	3,552	38	1·06	743
1925	Institute	42	..	..	27	583	6	1·02	219	625	6	0·96	246
	Centres	187	..	..	5	3,569	28	0·78	393	3,756	28	0·78	398
	Combined	229	..	..	32	4,152	34	0·81	612	4,381	34	0·79	644
1926	Institute	60	..	..	51	588	5	0·87	440	648	5	0·76	491
	Centres	287	..	..	20	3,861	25	0·64	322	4,148	25	0·60	342
	Combined	347	..	..	71	4,449	30	0·67	762	4,796	30	0·60	833
1927	Institute	51	..	..	37	678	6	0·88	468	729	6	0·82	505
	Centres	303	..	..	31	3,857	25	0·64	490	4,160	25	0·62	521
	Combined	354	..	..	68	4,535	31	0·68	958	4,889	31	0·61	1,026
1928	Institute	39	..	..	40	679	6	0·88	418	718	6	0·83	458
	Centres	216	..	..	27	4,166	34	0·81	467	4,382	34	0·77	494
	Combined	255	..	..	67	4,845	40	0·82	885	5,100	40	0·78	952
1929	Institute	35	..	..	43	650	5	0·77	522	685	5	0·73	565
	Centres	286	..	..	19	4,621	43	0·93	507	4,907	43	0·89	526
	Combined	321	..	..	62	5,271	48	0·91	1,029	5,592	48	0·89	1,091
1930	Institute	26	..	..	34	710	..	..	401	736	..	..	435
	Centres	314	1	0·29	10	4,692	50	1·07	92	5,006	51	1·01	102
	Combined	340	..	..	44	5,402	50	0·93	493	5,742	51	0·89	537
1931	Institute	41	..	..	42	1,020	3	0·29	394	1,061	3	0·28	436
	Centres	328	..	..	3	5,670	40	0·70	80	5,998	40	0·68	83
	Combined	369	..	..	45	6,690	43	0·64	474	7,059	43	0·60	519
	Grand Total ..	2,586	2	0·07	489	41,601	339	0·81	6,709	44,187	341	0·77	7,198

TABLE II.

A general classification of patients attending the Institute and its Centres during 1931.

Institute.

	Europeans					Asiatics				
	Class of case treated					Class of case treated				
	I	II	III	IV	Total	I	II	III	IV	Total
Number fully treated	22	3	15	1	41	20	301	2/585	1/114	3/1020
Percentage to total	53·6	7·3	36·6	2·4	..	1·08	29·5	57·4	11·2	..
Total deaths	..	..	..	..	..	..	..	2	1	3
Total death rate per cent. ..	..	..	..	..	..	..	..	·34	·87	·29
Percentage of health returns					100					95·5
Number not fully treated (presumably at risk)					*Nil.*					195
Advice Cases (not at risk)					42					394
Cases excluded from the statistical tables for other reasons					..					..
Total					42					589
Grand Total					83					1,609

TABLE II-a.

A general classification of patients attending the Institute and its Centres during 1931.

Centres.

	Europeans					Asiatics				
	Class of case treated					Class of case treated				
	I	II	III	IV	Total	I	II	III	IV	Total
Number fully treated	190	73	65	..	328	177	2/1785	22/2672	16/1036	40/5670
Percentage to total	57·9	22·3	19·8	..	..	3·1	31·4	47·1	18·2	..
Total deaths	..	..	..	..	..	..	2	22	16	40
Total death rate per cent. ..	..	..	..	..	..	..	0·11	0·82	1·5	0·70
Percentage of health returns					85·9					72·08
Number not fully treated (presumably at risk)					17					618
Advice Cases (not at risk)					3					80
Cases excluded from the statistical tables for other reasons					..					..
Total					20					698
Grand Total					348					6,368

TABLE III.

Classification of patients according to position of wounds, interposition of clothing and probability of rabies in the biting animal.

Numerators—Deaths. Institute *Denominators*—cases.

A—Animal proved rabid.
B—Animal certified rabid.
C—Animal probably rabid according to history.
D—History of animal unknown.
E—Animal probably not rabid according to history.

	Europeans							Asiatics							Total	Death rate per cent.
	A	B	C	D	E	Total	Death rate per cent.	A	B	C	D	E	Total	Death rate per cent.		
Bitten on face	..	..	..	1	..	1	..	..	..	1/18	16	..	1/34	2·9	1/35	2·8
Bitten on trunk and extremities (bare skin)	4	..	6	3	..	13	..	23	29	2/185	406	4	2/647	0·3	2/660	0·3
Bitten on trunk and extremities (through clothing) ..	2	..	..	3	..	5	..	9	4	60	245	2	320	..	325	..
"Licks" on any part of the body	12	9	1	..	..	22	..	7	2	2	8	..	19	..	41	..
Total ..	18	9	7	7	..	41	..	39	35	3/265	675	6	3/1020	0·29	3/1061	0·28
Percentage to total ..	43·9	21·9	17·0	17·1		..	..	3·9	3·6	25·9	66·1	0·5	..	..	..	..
Percentage death rate ..	..	..	..	..	..	..	..	..	..	1·1	..	..	0·29	..	..	..

TABLE III-a.

Classification of patients according to position of wounds, interposition of clothing and probability of rabies in the biting animal.

Numerators—Deaths. *Denominators*—Cases.

Centres.

A—Animal proved rabid.
B—Animal certified rabid.
C—Animal probably rabid according to history.
D—History of animal unknown.
E—Animal probably not rabid according to history.

—	Europeans							Asiatics							Total	Death Rate per cent.
	A	B	C	D	E	Total	Death rate per cent.	A	B	C	D	E	Total	Death rate per cent.		
Bitten on face	..	..	2	1	..	3	..	3	1/20	12/236	2/32	..	15/291	5·1	15/294	5·1
Bitten on trunk and extremities (bare skin)	8	9	20	20	..	57	..	31	4/245	18/2,386	1/678	..	23/3,340	0·68	23/3,397	0·67
Bitten on trunk and extremities (through clothing).. ..	2	8	23	23	..	56	..	9	176	2/1,193	444	..	2/1,822	0·10	2/1,878	0·10
'Licks' on any part of the body	87	65	57	3	..	212	..	42	69	79	27	..	217	..	429	..
Total ..	97	82	102	47	..	328	..	85	5/510	32/3,894	3/1,181	..	40/5,670	0·70	40/5,998	0·68
Percentage to total ..	29·5	25·0	31·0	14·5	..	..	..	1·14	8·9	68·6	20·9	..	..	..	..	..
Percentage death rate ..	..	..	..	..	..	..	..	..	0·98	0·82	0·25	..	..	..	..	..

TABLE IV.

Age and sex distribution of patients treated at the Institute.

Numerator—Deaths. *Denominators*—Cases.

Institute.

Age in years	Europeans				Asiatics				Europeans and Asiatics combined	
	Males	Females	Total	Death rate per cent.	Males	Females	Total	Death rate per cent.	Total	Death rate per cent.
0–4	1	2	3	..	18	12	30	..	33	..
5–9	4	3	7	..	1/97	40	1/137	0·73	1/144	0·69
10–14	5	1	6	..	1/130	30	1/160	0·62	1/166	0·60
15–19	0	3	3	..	61	16	77	..	80	..
20–29	5	1	6	..	187	1/28	1/215	0·46	1/221	0·45
30–39	4	3	7	..	161	24	185	..	192	..
40–49	3	1	4	..	95	34	129	..	133	..
50–59	3	..	3	..	38	15	53	..	56	..
60–69	..	1	1	..	13	19	32	..	33	..
70 and over	1	..	1	..	1	1	2	..	3	..
Total ..	26	15	41	..	2/799	1/218	3/1,020	0·29	3/1,061	0·28

TABLE IV-a.

Age and sex distribution of patients treated at Centres.

Numerator—Deaths. Centres. *Denominators*—Cases.

Age in years	Europeans				Asiatics				Europeans and Asiatics combined	
	Males	Females	Total	Death rate per cent.	Males	Females	Total	Death rate per cent.	Total	Death rate per cent.
0–4	13	9	22	..	3/330	2/70	5/400	1·2	5/422	1·1
5–9	8	17	25	..	9/805	256	9/1,061	0·84	9/1,086	0·82
10–14	13	5	18	..	7/670	1/147	8/817	0·97	8/835	0·95
15–19	12	11	23	..	2/419	86	2/505	0·39	2/528	0·37
20–29	83	30	113	..	3/882	2/193	5/1,075	0·46	5/1,188	0·42
30–39	51	30	81	..	1/806	2/162	3/968	0·30	3/1,049	0·29
40–49	20	12	32	..	4/319	124	4/443	0·90	4/475	0·84
50–59	7	5	12	..	3/197	83	3/280	1·08	3/292	1·03
60–69	..	1	1	..	1/71	27	1/98	1·01	1/99	1·01
70 and over	..	1	1	..	16	7	23	..	24	..
Total ..	207	121	328	..	33/4,515	7/1,155	40/5,670	0·70	40/5,998	0·66

TABLE V.

Classification according to number and position of wounds.

Numerators—Deaths. *Denominators*—Cases.

Europeans.

Institute.

Position	Number of wounds																Total	Percentage death rate
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over		
Leg	..	2	3	...	3	..	..	..	..	..	..	..	..	..	..	..	8	
Trunk	..	1	..	1	..	..	..	..	..	..	..	..	..	..	..	..	2	
Arm	22	2	3	1	..	..	1	..	..	..	..	..	..	..	..	..	29	
Multiple	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	1	
Face	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	1	
Multiple face ..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	
Total ..	22	5	7	3	3	..	1	..	..	..	..	..	..	..	..	..	41	
Percentage death rate.			*Nil.*															

Separate table for Europeans and Asiatics ; also for Institute and Centres :—

(V) Europeans—Institute.
(V-a) ,, —Centres.
(V-b) Asiatics —Institute.
(V-c) ,, —Centres.

TABLE V-a.

Classification according to number and position of wounds.

Numerators—Deaths. *Denominators*—Cases.

Europeans.

Centres.

Position	Number of wounds																Total	Percentage death rate
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over		
Leg	..	14	16	5	6	4	3	1	1	1	..	..	..	..	..	..	51	
Trunk	..	2	1	..	..	1	..	..	..	..	..	..	..	..	..	..	4	
Arm	212	17	17	7	6	2	2	..	..	..	2	..	..	..	..	..	265	
Multiple	..	..	..	..	1	1	2	..	1	..	..	..	..	..	..	..	5	
Face	..	..	..	1	..	..	..	..	1	..	..	..	..	..	..	..	2	
Multiple face ..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	1	
Total ..	212	33	35	13	13	8	7	1	3	1	2	..	..	..	..	..	328	
Percentage death rate.					*Nil.*													

Separate table for Europeans and Asiatics; also for Institute and Centres:—

(V) Europeans—Institute.
(V-a) „ —Centres.
(V-b) Asiatics —Institute.
(V-c) „ —Centres.

TABLE V-b.

Classification according to number and position of wounds.

Numerators—Deaths. *Denominators*—Cases.

Asiatics.

Institute.

Position	Number of wounds																Total	Percentage death rate
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over		
Leg	..	169	273	1/124	1/99	39	20	13	3	3	..	..	..	..	..	..	2/743	0·26
Trunk	..	5	3	3	..	..	1	1	..	..	..	..	..	..	..	..	13	..
Arm	19	57	54	24	16	10	12	3	3	1	3	2	1	..	..	2	207	..
Multiple	..	..	..	3	1	5	5	4	1	2	..	1	..	..	..	1	23	..
Face	..	3	9	1/2	5	4	1	1	2	..	..	..	..	..	..	..	1/27	3·7
Multiple face ..	..	..	1	1	..	..	..	4	..	..	..	1	..	..	..	..	7	..
Total ..	19	234	340	2/157	1/121	58	39	26	9	6	3	4	1	..	..	3	3/1,020	0·29
Percentage death rate ..	..	..	..	1·2	0·82	..	..	..	..	..	..	..	..	..	..	..	..	

Separate table for Europeans and Asiatics; also for Institute and Centres:—
(V) Europeans—Institute.
(V-a) ,, —Centres.
(V-b) Asiatics—Institute.
(V-c) ,, —Centres.

TABLE V-c.

Classification according to number and position of wounds.

Numerators—Deaths. *Denominators*—Cases.

Asiatics.

Centres.

Position	Number of wounds																Total	Percentage death rate
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over		
Leg	..	2/611	3/1,076	1/686	2/600	184	1/132	38	1/34	18	14	3	1	3	3	3	10/3,406	0·29
Trunk	..	38	1/90	57	33	19	11	5	7	3	3	..	..	..	..	..	1/266	0·37
Arm	219	279	358	3/162	2/85	2/86	1/72	2/43	42	13	25	5	2	..	2	1/9	11/1,402	0·78
Multiple	..	..	32	40	38	42	35	29	1/24	14	1/14	6	12	1/4	6	9	3/305	0·98
Face	..	47	3/51	3/41	1/25	1/8	1/5	7	2	..	3	..	..	..	..	..	9/189	4·7
Multiple face ..	..	1	1/12	1/11	14	23	1/10	1/7	6	..	1/4	5	1/4	..	1	4	6/102	5·8
Total ..	219	2/976	8/1,619	8/997	5/795	3/362	4/265	3/129	2/115	48	2/63	19	1/19	1/7	12	1/25	40/5,670	0·70
Percentage death rate	..	0·20	0·49	0·80	0·62	0·83	1·1	2·3	1·7	..	3·1	..	5·2	142	...	4·0	..	0·70

Separate table for Europeans and Asiatics; also for Institute and Centres—
(V) Europeans—Institute.
(V-a) " —Centres.
(V-b) Asiatics—Institute.
(V-c) " —Centres.

TABLE VI.

Classification according to number and depth of wounds and interposition of clothing.

Numerators—Deaths. Europeans. *Denominators*—Cases.

Institute.

Deep Bare	Number of wounds																Total	Percentage death rate
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over		
+ +	..	3	3	..	2	..	1	..	..	..	..	..	..	..	..	..	9	..
+ —	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	1	..
— +	..	1	2	2	..	..	..	..	..	..	..	..	..	..	..	..	5	..
— —	22	1	2	..	1	..	..	..	..	..	..	..	..	..	..	..	26	..
Total ..	22	5	7	3	3	..	1	..	..	..	..	..	..	..	..	..	41	..
Percentage death rate.				*Nil.*														

TABLE VI-a.

Classification according to number and depth of wounds and interposition of clothing.

Numerators—Deaths. Europeans. *Denominators*—Cases.

Centres.

Deep Bare	Number of wounds																Total	Percentage death rate
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over		
+ +	..	6	9	5	5	2	1	..	1	..	1	..	..	..	..	..	30	..
+ —	..	3	2	1	3	..	3	..	..	..	..	..	..	..	..	..	12	..
— +	212	14	18	6	4	2	2	..	..	..	1	..	..	..	..	..	259	..
— —	..	10	6	1	1	4	1	1	2	1	..	..	..	..	..	..	27	..
Total ..	212	33	35	13	13	8	7	1	3	1	2	..	..	..	..	..	328	..
Percentage death rate.			*Nil.*															

TABLE VI-b.

Classification according to number and depth of wounds and interposition of clothing.

Numerators—Deaths. Asiatics. *Denominators*—Cases.

Institute.

Deep Bare	Number of wounds 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over	Total	Percentage death rate
+ +	..	120	156	2/83	1/71	40	27	25	5	5	2	4	..	..	..	3	3/541	0·55
+ —	..	26	38	25	13	7	10	1	3	..	1	..	1	..	..	..	125	..
— +	19	44	48	28	15	4	..	..	..	1	..	..	..	..	..	..	159	..
— —	..	44	98	21	22	7	2	..	1	..	..	..		..	..	..	195	..
Total.	19	234	340	2/157	1/121	58	39	26	9	6	3	4	1	..	..	3	3/1020	0·29
Percentage death rate.	..	..	..	1·2	0·82	..	..	..	..	..	..	..	..	..	..	..	..	..

TABLE VI-c.

Classification according to number and depth of wounds and interposition of clothing.

Numerators—Deaths. Asiatics. *Denominators*—Cases.

Centres.

Deep Bare	Number of wounds 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over	Total	Percentage death rate
+ +	..	2/345	6/633	7/419	4/390	3/185	4/134	2/67	2/76	33	2/34	11	1/14	1/5	7	1/19	35/2372	1·05
+ —	..	110	283	233	1/185	78	59	29	20	6	16	4	4	2	4	3	1/1036	0·09
— +	219	352	2/427	223	138	69	38	1/23	12	6	10	2	..	..	1	2	3/1522	0·19
— —	..	169	276	1/122	82	30	34	10	7	3	3	2	1	..	..	1	1/740	0·14
Total.	219	2/976	8/1619	8/997	5/795	3/362	4/265	3/129	2/115	48	2/63	19	1/19	1/7	12	1/25	40/5670	0·70
Percentage death rate.	..	0·20	0·49	0·80	0·62	0·83	1·1	2·3	1·7	..	3·1	..	5·2	14·2	..	4·0	..	0·70

TABLE VII.

Classification according to number of wounds and lateness of arrival.

Numerators—Deaths. Europeans. *Denominators*—Cases.

Institute.

Days late	Number of wounds																Total	Percentage death rate
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over		
0 ..	..	1	2	2	1	..	..	..	..	..	..	..	..	..	..	..	6	..
1 ..	1	1	3	1	2	..	..	..	..	..	..	..	..	..	..	..	8	..
2 ..	1	1	1		..	..	..	..	..	..	..	..	..	..	..	..	3	..
3 ..	1	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	2	..
4 ..	6	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	6	..
5 ..	5	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	5	..
6 ..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..
7 ..	4	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	4	..
8 to 14 ..	2	1	1	..	..	..	..	..	..	..	..	..	..	..	..	..	4	..
15 to 21 ..	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	2	..
22 to 28 ..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
29 to 35 ..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
36 to 42 ..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
43 to 49 ..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
50 and over ..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Total ..	22	5	7	3	3	..	1	..	..	..	..	..	..	..	..	..	41	..
Percentage death rate.						*Nil.*												

TABLE VII-a.

Classification according to number of wounds and lateness of arrival.

Numerators—Deaths. Europeans. *Denominators*—Cases.

Centres.

Days late	Number of wounds																Total	Percentage death rate
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over		
0 ..	28	3	1	2	2	..	1	..	..	1	..	..	..	..	..	..	38	..
1 ..	36	4	7	5	4	3	2	1	1	..	..	..	..	..	..	..	63	..
2 ..	38	6	6	2	1	1	..	..	1	..	..	..	..	..	..	..	55	..
3 ..	12	5	5	1	1	1	2	..	1	..	..	..	..	..	..	..	28	..
4 ..	15	5	5	..	3	2	1	..	..	..	1	..	..	..	..	..	32	..
5 ..	11	2	2	..	2	..	..	..	..	..	..	..	..	..	..	..	17	..
6 ..	12	..	3	1	..	..	..	..	..	..	..	..	..	..	..	..	16	..
7 ..	16	5	2	2	..	..	..	..	..	..	1	..	..	..	..	..	26	..
8 to 14 ..	41	2	2	..	..	1	1	..	..	..	..	..	..	..	..	..	47	..
15 to 21 ..	3	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	4	..
22 to 28 ..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..
29 to 35 ..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
36 to 42 ..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
43 to 49 ..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..
50 and over ..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Total ..	212	33	35	13	13	8	7	1	3	1	2	..	..	..	..	..	328	..
Percentage death rate.						*Nil.*												

TABLE VII-b.

Classification according to number of wounds and lateness of arrival.

Numerators—Deaths. Asiatics. *Denominators*—Cases.

Institute.

Days late	Number of wounds																Total	Percentage death rate
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over		
0 ..	..	36	56	1/28	26	10	8	5	3	2	..	2	..	..	..	1	1/177	0·56
1 ..	9	80	138	67	44	26	15	9	2	1	2	2	1	..	..	1	397	..
2 ..	2	23	43	15	20	10	4	2	2	..	..	..	..	..	..	..	121	..
3 ..	..	21	17	6	7	3	5	2	..	..	..	..	..	..	..	..	61	..
4 ..	1	8	19	9	9	2	2	1	..	1	..	..	..	..	..	..	52	..
5 ..	4	13	11	11	2	2	2	3	..	1	..	..	..	..	..	..	49	..
6 ..	..	6	6	1	5	1	..	..	2	..	..	..	..	..	..	..	21	..
7 ..	..	5	4	3	..	..	2	..	..	..	..	..	..	..	..	..	14	..
8 to 14 ..	1	25	21	6	3	2	..	1	..	1	..	..	..	..	..	1	62	..
15 to 21 ..	1	10	13	..	1/3	1	1	2	..	..	..	..	..	..	..	..	1/31	3·2
22 to 28 ..	..	5	..	3	..	..	..	..	..	..	..	..	..	..	..	..	8	..
29 to 35 ..	..	1	4	2	2	1	..	1	..	..	..	..	..	..	..	..	11	..
36 to 42 ..	..	..	1	1	..	..	..	..	..	..	..	..	..	..	..	..	2	..
43 to 49 ..	..	1	2	1/2	..	..	..	..	..	..	..	..	..	..	..	..	1/5	20·0
50 and over ..	1	..	5	3	..	..	..	..	..	..	..	..	..	..	..	..	9	..
Total ..	19	234	340	2/157	1/121	58	39	26	9	6	2	4	1	..	..	3	3/1,020	0·29
Percentage death rate.	..	..	..	1·2	0·82	..	..	..	..	..	..	..	..	..	..	..	..	0·29

TABLE VII-c.

Classification according to number of wounds and lateness of arrival.

Numerators—Deaths. Asiatics. *Denominators*—Cases.

Centres.

Days late	Number of wounds																Total	Percentage death rate
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 and over		
0 ..	21	25	1/56	29	26	26	21	14	7	3	6	1	1/1	..	..	..	2/236	0·84
1 ..	40	1/135	3/218	145	108	1/59	1/53	1/25	1/18	9	1/9	3	4	1	1	2	9/830	1·08
2 ..	30	1/178	2/308	1/167	1/165	66	39	2/24	21	7	14	4	1	3	1	4	7/1,032	0·68
3 ..	17	157	262	2/155	116	1/47	1/29	16	14	5	1/5	2	..	1/1	..	1/7	7/833	0·81
4 ..	13	118	199	2/143	95	1/27	1/18	12	13	4	3	1	1	..	2	5	4/654	0·61
5 ..	21	90	1/147	1/94	2/68	28	22	6	7	2	2	3	1	1	..	3	4/495	0·88
6 ..	18	64	119	72	47	14	19	6	4	2	1	1	..	..	1	1	369	..
7 ..	13	67	113	58	47	12	11	3	3	3	1	..	..	..	3	..	334	..
8 to 14 ..	32	99	1/130	2/109	1/96	72	33	14	1/9	4	2	1	..	..	..	1	5/602	0·83
15 to 21 ..	3	28	44	17	1/22	7	1/3	2	3	..	1	1	1	..	..	..	2/132	1·5
22 to 28 ..	3	3	3	1	2	3	1	..	..	..	..	..	3	1	..	..	20	..
29 to 35 ..	..	8	12	5	2	..	2	3	3	3	12	1	6	..	2	..	59	..
36 to 42 ..	3	..	..	..	1	..	..	..	6	6	7	..	1	..	..	1	25	..
43 to 49 ..	2	1	4	2	..	1	2	3	6	..	..	1	..	..	1	..	23	..
50 and over ..	3	3	4	..	..	..	12	1	1	..	..	..	..	..	1	1	26	..
Total ..	219	2/976	8/1,619	8/997	5/795	3/362	4/265	3/129	2/115	48	2/63	19	1/19	1/7	12	1/25	40/5,670	0·70
Percentage death rate. ..	..	0·20	0·49	0·80	0·62	0·83	1·1	2·3	1·7	..	3·1	..	5·2	14·2	..	4·0	..	0·70

TABLE VIII.

Classification according to position and depth of wounds, and interposition of clothing.

Numerators—Deaths. Europeans. *Denominators*—Cases.

Institute.

Deep Bare	Leg	Trunk	Arm	Multiple	Face	Multiple face	Total	Percentage death rate
+ + ..	5	..	3	1	..	..	9	..
+ — ..	..	1	..	..	..	..	1	..
— + ..	..	..	4	..	1	..	5	..
— — ..	3	1	22	..	..	..	26	..
Total ..	8	2	29	1	1	..	41	..
Percentage death rate.			*Nil.*					

TABLE VIII-a.

Classification according to position and depth of wounds, and interposition of clothing.

Numerators—Deaths. Europeans. *Denominators*—Cases.

Centres.

Deep Bare	Leg	Trunk	Arm	Multiple	Face	Multiple face	Total	Percentage death rate
+ + ..	8	1	16	2	2	1	30	..
+ — ..	9	3	..	..	..	..	12	..
— + ..	21	..	236	2	..	..	259	..
— — ..	13	..	13	1	..	..	27	..
Total ..	51	4	265	5	2	1	328	..
Percentage death rate.			*Nil.*					

TABLE VIII-b.

Classification according to position and depth of wounds, and interposition of clothing.

Numerators—Deaths. Asiatics. *Denominators*—Cases.

Institute.

Deep Bare	Leg	Trunk	Arm	Multiple	Face	Multiple face	Total	Percentage death rate
+ + ..	2/350	4	131	22	1/27	7	3/541	0·55
+ — ..	116	3	6	..	..	..	125	..
— + ..	101	1	56	1	..	..	159	..
— — ..	176	5	14	..	..	..	195	..
Total ..	2/743	13	207	23	1/27	7	3/1,020	0·29
Percentage death rate.	0·26	..	..	..	3·7	..	..	0·29

TABLE VIII-c.

Classification according to position and depth of wounds, and interposition of clothing.

Numerators—Deaths. Asiatics. *Denominators*—Cases.

Centres.

Deep Bare	Leg	Trunk	Arm	Multiple	Face	Multiple face	Total	Percentage death rate
+ + ..	8/1,250	1/68	9/625	3/192	8/151	6/86	35/2,372	1·05
+ — ..	1/734	77	175	37	10	3	1/1,036	0·09
— + ..	1/883	43	1/508	59	1/20	9	3/1,522	0·19
— — ..	539	78	1/94	17	8	4	1/740	0·14
Total ..	10/3,406	1/266	11/1,402	3/305	9/189	6/102	40/5,670	0·70
Percentage death rate.	0·29	0·37	0·78	0·98	4·07	5·8	..	0·70

TABLE IX.

Classification according to position of wounds and lateness of arrival.

Numerators—Deaths. Europeans. *Denominators*—Cases.

Institute.

Days late	Leg	Trunk	Arm	Multiple	Face	Multiple face	Total	Percentage death rate
0 ..	4	..	1	..	1	..	6	..
1	3	1	4	..	..	..	8	..
2 ..	..	..	3	..	..	..	3	..
3 ..	..	..	2	..	..	..	2	..
4 ..	..	..	6	..	..	..	6	
5 ..	..	..	5	..	..	..	5	..
6 ..	..	1	..	..	..	..	1	..
7 ..	..	..	4	..	..	..	4	..
8 to 14 ..	1	..	2	1	..	..	4	..
15 to 21 ..	..	..	2	..	..	..	2	..
22 to 28 ..	..	..	..	..	..	..	..	..
29 to 35 ..	..	..	..	..	..	..	..	..
36 to 42 ..	..	..	..	..	..	..	..	..
43 to 49 ..	..	..	..	..	..	..	..	..
50 and over ..	..	..	..	..	..	..	..	..
Total ..	8	2	29	1	1	..	41	..
Percentage death rate.				*Nil*				

TABLE IX-a.

Classification according to position of wounds and lateness of arrival.

Numerators—Deaths. Europeans. *Denominators*—Cases.

Centres.

Days late	Leg	Trunk	Arm	Multiple	Face	Multiple face	Total	Percentage death rate
0 ..	6	1	29	1	1	..	38	..
1 ..	12	2	47	1	1	..	63	..
2 ..	11	..	44	..	..	..	55	..
3 ..	8	..	19	1	..	..	28	..
4 ..	5	..	25	2	..	..	32	..
5 ..	3	..	14	..	..	..	17	..
6 ..	1	1	14	..	..	..	16	..
7 ..	1	..	24	..	..	1	26	..
8 to 14 ..	3	..	44	..	..	..	47	..
15 to 21 ..	..	..	4	..	..	..	4	..
22 to 28	..	..	1	..	..	..	1	..
29 to 35 ..	..	..	..	..	..	..	..	..
36 to 42 ..	..	..	..	..	..	..	..	..
43 to 49 ..	1	..	..	..	..	..	1	..
50 and over..	..	..	..	..	..	..	..	..
Total ..	51	4	265	5	2	1	328	..
Percentage death rate.				*Nil*				

TABLE IX-b.

Classification according to position of wounds and lateness of arrival.

Numerators—Deaths. Asiatics. *Denominators*—Cases.

Institute.

Days late	Leg	Trunk	Arm	Multiple	Face	Multiple face	Total	Percentage death rate
0 ..	133	1	26	6	1/8	3	1/177	0·56
1 ..	287	7	78	11	13	1	397	..
2 ..	95	..	23	2	1	..	121	..
3 ..	41	1	16	2	1	..	61	..
4 ..	35	2	11	2	2	..	52	..
5 ..	35	..	13	..	1	..	49	..
6 ..	13	..	8	..	..	..	21	..
7 ..	10	..	4	..	..	..	14	..
8 to 14 ..	44	1	16	..	..	1	62	..
15 to 21 ..	1/22	1	6	..	1	1	1/31	3·2
22 to 28 ..	7	..	1	..	..	..	8	..
29 to 35 ..	9	..	1	..	..	1	11	..
36 to 42 ..	1	..	1	..	..	..	2	..
43 to 49 ..	1/4	..	1	..	..	..	1/5	20·0
50 and over ..	7	..	2	..	..	..	9	..
Total ..	2/743	13	207	23	1/27	7	3/1,020	0·29
Percentage death rate.	0·26	..	..	..	3·7	..	..	..

TABLE IX-c.

Classification according to position of wounds and lateness of arrival.

Numerators—Deaths. *Denominators*—Cases.

Asiatics.

Centres.

Days late	Leg	Trunk	Arm	Multiple	Face	Multiple face	Total	Percentage death rate
0 ..	155	6	51	14	1/5	1/5	2/236	0·84
1 ..	5/446	46	1/221	67	38	3/12	9/830	1·08
2 ..	2/585	1/53	2/274	67	2/37	16	7/1032	0·68
3 ..	451	51	2/225	2/45	3/40	21	7/833	0·81
4 ..	335	45	1/198	32	1/24	2/20	4/654	0·61
5 ..	2/349	34	2/73	29	6	4	4/495	0·88
6 ..	248	5	72	22	13	9	369	..
7 ..	266	6	46	6	5	5	334	..
8 to 14 ..	390	12	2/165	1/13	2/16	6	5/602	0·83
15 to 21 ..	1/80	4	1/39	4	4	1	2/132	1·5
22 to 28 ..	9	1	8	2	..	..	20	..
29 to 35 ..	46	1	10	1	..	1	59	..
36 to 42 ..	25	..	..	..	..	..	25	..
43 to 49 ..	10	..	11	1	1	..	23	..
50 and over ..	11	2	9	2	..	2	26	..
Total ..	10/3406	1/266	11/1402	3/305	9/189	6/102	40/5670	0·70
Percentage death rate.	0·29	0·37	0·78	0·98	4·07	5·8	..	..

TABLE X.

Deaths from hydrophobia amongst persons treated in 1931.

Europeans.

Institute.

Serial Number	Case Number	Number of days from commencement of treatment to onset of symptoms	Sex	Age in years	Biting animal		Number and position of wounds					Wounds				Number of days late	Duration of illness in days	Interval between bite and death in days	Class of cases	Treatment		Serial Number
					Kind	Class	Leg	Trunk	Arm	Face	Total	Deep	Extensive	Bare skin	Uncauterised					Type	Duration in days	
← Nil →																						

TABLE X-a.

Deaths from hydrophobia amongst persons treated in 1931.

Europeans.

Centres.

Serial Number	Case Number	Number of days from commencement of treatment to onset of symptoms	Sex	Age in years	Biting animal		Number and position of wounds					Wounds				Number of days late	Duration of illness in days	Interval between bite and death in days	Class of cases	Treatment		Serial Number
					Kind	Class	Leg	Trunk	Arm	Face	Total	Deep	Extensive	Bare skin	Uncauterised					Type	Duration in days	
← Nil →																						

TABLE X-b.

Deaths from hydrophobia amongst persons treated in.....

Asiatics.

Institute.

Serial Number	Case Number	Number of days from commencement of treatment to onset of symptoms	Sex	Age in years	Biting animal		Number and position of wounds					Wounds				Number of days late	Duration of illness in days	Interval between bite and death in days	Class of cases	Treatment		Serial Number
					Kind	Class	Leg	Trunk	Arm	Face	Total	Deep	Extensive	Bare skin	Uncauterised					Type	Duration in days	
1	592	21	M.	6	Dog.	C	3	..	..	..	3	+	+	+	+	45	2	68	III	33	14	
2	322	24	M.	12	,,	C	..	..	..	3	3	+	+	+	+	..	1	25	IV	33	14	
3	1297	42	F.	20	,,	C	4	..	..	..	4	+	+	+	+	16	1	59	III	33	14	

TABLE

Deaths from hydrophobia amongst

Asiatic

Centres

Serial Number	Case Number	Number of days from commencement of treatment to onset of symptoms	Sex	Age in years	Biting Animal		Number and of		
					Kind	Class	Leg	Trunk	Arm
								Died	*during*
1	30	1	M.	30	Jackal ..	C	..	..	..
2	126	1	M.	40	Dog ..	C	..	..	..
3	21	2	M.	38	Dog ..	C	2	..	1
4	60	6	F.	60	Dog ..	C	3	..	..
5	196	10	F.	40	Jackal ..	C	..	..	..
6	43	13	F.	10	Dog ..	A	5	..	2
							Died within fifteen days		
1	81	15	M.	25	Dog ..	D	..	..	2
2	105	16	M.	10	Dog ..	C	..	..	7
3	253	16	M.	1½	Dog ..	C	..	..	..
4	206	16	M.	28	Dog ..	C	4	..	..
5	238	17	M.	6	Dog ..	B	..	..	..
6	788	18	M.	4	Dog ..	C	..	..	3
7	40	18	M.	40	Dog ..	C	..	..	..
8	306	24	M.	10	Dog ..	C	..	..	7
9	123	24	M.	8	Dog ..	C	..	..	4
10	203	25	F.	35	Dog ..	B	..	..	15
11	112	25	M.	45	Jackal ..	C	1	..	..
12	13	26	M.	50	Hyena ..	C	..	..	7
13	238	26	M.	7	Dog ..	C	..	..	5
							Died after fifteen days		
1	487	27	M.	14	Dog ..	D	8	..	..
2	163	27	F	10	Dog ..	C	..	..	..
3	10	29	M.	42	Dog ..	C	..	..	8
4	6	29	M.	18	Dog ..	B	2	..	11
5	158	29	M.	46	Jackal ..	D	..	..	..
6	647	30	M.	6	Dog ..	C	1	..	..
7	333	30	M.	7	Dog ..	C	..	..	3

X-c

persons treated in 1931

position wounds		Wounds				Number of days late	Duration of illness in days	Interval between bite and death in days	Class of cases	Treatment	
Face	Total	Deep	Extensive	Bare skin	Uncauterised					Type	Duration in days
Treatment											
..	..	..	..	..	..	45	1	47	III	16	1
..	..	..	..	..	..	275	1	277	III	16	1
..	3	+	+	+	+	40	1	43	III	16	2
..	3	+	+	+	+	54	1	61	III	16	6
2	2	+	+	+	+	4	2	16	III	16	10
..	7	+	+	+	+	45	1	59	III	16	13
after treatment											
1	3	+	+	+	+	4	2	21	IV	16	14
..	7	−	−	+	+	2	4	22	III	16	14
3	3	+	+	+	+	14	1	31	IV	16	14
..	4	+	+	−	+	21	2	39	III	16	14
2	2	−	−	+	+	2	2	22	IV	16	14
..	3	+	+	+	+	12	1	31	III	16	14
2	2	+	+	+	+	0	1	19	IV	16	14
..	7	+	+	+	+	2	2	28	III	16	14
..	4	+	+	+	+	5	2	31	III	16	14
..	15	+	+	+	+	3	2	30	III	16	14
1	2	+	+	+	+	1	2	28	IV	16	14
3	10	+	+	+	+	1	1	28	IV	16	14
..	5	+	+	+	+	1	3	30	III	16	14
after treatment											
..	8	+	+	+	+	1	3	31	III	16	14
5	5	+	+	+	+	3	2	32	IV	16	14
4	12	+	+	+	+	0	1	30	IV	16	14
..	13	+	+	+	+	3	3	35	III	16	14
4	4	+	+	+	+	2	2	33	IV	16	14
..	1	+	+	+	+	1	2	33	IV	16	14
..	3	+	+	+	+	5	2	37	III	16	14

TABLE

Serial Number	Case Number	Number of days from commencement of treatment to onset of symptoms	Sex	Age in years	Biting Animal		Number and of		
					Kind	Class	Leg	Trunk	Arm
8	79	32	M.	11	Dog ..	C	..	1	9
9	143	34	F.	25	Dog ..	C	..	3	2
10	156	41	M.	28	Dog ..	C	4	4	..
11	130	42	F	1¼	Jackal ..	C	..	..	..
12	1017	51	M.	8	Dog ..	C	3	..	..
13	38	63	M.	5	Dog ..	C	2	..	..
14	966	67	M.	11	Dog ..	C	6	..	..
15	390A	67	M.	13	Dog ..	B	2	..	..
16	378	70	M.	15	Jackal ..	C	..	..	3
17	33	77	M.	50	Dog ..	C	..	2	..
18	348	79	F.	3	Dog ..	C	..	..	..
19	315	83	M.	6	Dog ..	C	..	..	3
20	27	90	M.	7	Dog ..	C	2	..	..
21	347	93	M.	50	Dog ..	D	..	..	5
22	203	108	F.	30	Dog ..	C	3	..	..
23	325	111	M.	2	Dog ..	C	..	..	..
24	917	173	M.	60	Dog ..	C	1	..	..
25	205	205	M.	14	Dog ..	C	..	..	..
26	191	220	M.	33	Dog ..	C	..	..	4
27	64	298	F.	23	Dog ..	C	..	..	6

X-c—*contd.*

position wounds		Wounds								Treatment	
Face	Total	Deep	Extensive	Bare skin	Uncauterised	Number of days late	Duration of illness in days	Interval between bite and death in days	Class of cases	Type	Duration in days
..	10	+	+	+	+	3	2	38	III	16	14
2	7	+	+	+	+	1	2	37	IV	16	14
..	8	+	+	+	+	8	2	51	III	16	14
3	3	+	+	+	+	4	1	47	IV	16	14
..	3	+	+	+	+	2	1	54	III	16	14
..	2	—	—	+	+	5	3	71	II	14	7
..	6	+	+	+	+	1	1	69	III	16	14
..	2	+	+	+	+	1	2	70	III	16	14
3	6	+	+	+	+	4	3	78	IV	16	14
..	2	+	+	+	+	2	2	81	III	16	14
3	3	+	+	+	+	3	1	83	IV	16	14
..	3	—	—	—	+	3	2	88	II	14	7
..	2	+	+	+	+	1	2	93	III	16	14
..	5	+	+	+	+	4	2	99	III	16	14
..	3	+	+	+	+	5	2	115	III	16	14
6	6	+	+	+	+	3	2	116	IV	16	14
..	1	+	+	+	+	2	2	117	III	16	14
2	2	+	+	+	+	8	3	216	IV	16	14
..	4	+	+	+	+	10	2	232	III	16	14
..	6	+	+	+	+	15	3	316	III	16	14

TABLE

Classification of the animal which bit,

Numerators—Deaths.

Animal	Institute								
	Number treated			Percentage to total			Death rate per cent.		
	Europeans	Asiatics	Combined	Europeans	Asiatics	Combined	Europeans	Asiatics	Combined
Dog ..	41	3/964	3/1005	100	94·5	95·6	..	0·31	0·29
Jackal ..	..	10	10	..	0·98	0·94	..	..	..
Cat ..	..	17	17	..	1·6	1·6	..	..	..
Monkey ..	..	18	18	..	1·7	1·7	..	..	..
Fox ..	..	1	1	..	0·1	0·1	..	..	..
Man ..	..	..	..	..	..	..	..	..	..
Donkey ..	..	1	1	..	0·1	0·1	..	..	..
Horse ..	..	..	..	..	..	..	..	..	..
Buffalo ..	..	..	..	..	..	..	..	..	..
Hyena ..	..	..	..	..	..	..	..	..	..
Wolf ..	..	..	..	..	..	..	..	..	..
Cow ..	..	..	..	..	..	..	..	..	..
Goat ..	..	1	1	..	0·1	0·1	..	..	..
Leopard ..	..	..	..	..	..	..	..	..	..
Tiger ..	..	..	..	..	..	..	..	..	..
Bear ..	..	..	..	..	..	..	..	..	..
Unknown ..	..	8	8	..	0·8	0·8	..	..	..
Total ..	41	3/1020	3/1061	..	..	..	..	..	..

XI.

scratched or licked the patient.

Denominators—Cases.

Centres								
Number treated			Percentage to total			Death rate per cent.		
Europeans	Asiatics	Combined	Europeans	Asiatics	Combined	Europeans	Asiatics	Combined
315	35/5219	35/5534	96·03	92·04	92·2	..	0·67	0·65
..	4/373	4/373	..	6·5	6·2	..	1·07	1·07
5	11	16	1·5	0·19	0·26	..	..	..
2	9	11	0·60	0·15	0·18	..	..	..
..	14	14	..	0·24	0·23	..	..	..
6	10	16	1·8	0·17	0·26	..	..	..
..	7	7	..	0·12	0·11	..	..	..
..	3	3	..	0·05	0·05	..	..	..
..	11	11	..	0·19	0·18	..	..	..
..	1/5	1/5	..	0·01	0·08	..	20·0	20·0
..	2	2	..	0·03	0·03	..	..	..
..	3	3	..	0·05	0·05	..	..	..
..	..	..	..	..	..	..	..	..
..	1	1	..	0·01	0·01	..	..	..
..	1	1	..	0·01	0·01	..	..	..
..	1	1	..	0·01	0·01	..	..	..
..	..	..	..	..	..	..	..	..
328	40/5670	40/5998	..	..	..	..	..	..

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TABLE XII.

Number of patients treated by months.

	Institute			Centres			Institute and centres combined		
	Europeans	Asiatics	Total	Europeans	Asiatics	Total	Europeans	Asiatics	Total
January ..	1	99	100	40	492	532	41	591	632
February ..	3	92	95	28	449	477	31	541	572
March ..	10	113	123	39	546	585	49	659	708
April ..	4	107	111	29	496	525	33	603	636
May ..	4	96	100	31	456	487	35	552	587
June ..	5	90	95	19	523	542	24	613	637
July ..	5	66	71	20	485	505	25	551	576
August ..	..	79	79	35	433	468	35	512	547
September ..	..	53	53	29	425	454	29	478	507
October ..	4	72	76	17	443	460	21	515	536
November ..	..	69	69	20	467	487	20	536	556
December ..	5	84	89	21	455	476	26	539	565
Grand Total ..	41	1,020	1,061	328	5,670	5,998	369	6,690	7,059

TABLE XIII.

Statement showing brains submitted and examined for rabies.

Serial number	Animal	Number of brains examined	Positive	Negative	Unfit for examination
1	Dog	170	109	51	10
2	Cat	1	1	..	..
3	Jackal	1	1	..	..
4	Bullock	1	..	..	1
5	Rabbit	1	..	1	..
6	Guineapig	3	3	..	..
	Total ..	177	114	52	11

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